

CULTURABLE BACTERIAL ENDOPHYTES FROM
BERMUDAGRASS AND TAQMAN[®] REAL-TIME
PCR TO QUANTIFY *OPHIOSPHAERELLA*
HERPOTRICA

By

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PREFACE

Spring dead spot (SDS) is a devastating fungal disease of bermudagrass (*Cynodon dactylon* (L.) Pers. and *C. dactylon* X *C. transvaalensis* Burt-Davy). The causal agents are *Ophiosphaerella korrae* (J. C. Walker & A. M. Smith) Shoemaker & C. E Babcock, *O. herpotricha* (Fr.:Fr.) Pers., and *O. narmari* Wetzell, Hulbert & Tisserat. These *Ophiosphaerella* spp. are soilborne, root-infecting fungi that live off of plant derived nutrients. Traditional pathogen control methods including the use of resistant bermudagrass cultivars, fungicides, and specific cultivation practices, have found limited success in controlling SDS in Oklahoma and Kansas. Biological control agents against SDS have yet to be developed. In hopes of finding culturable bacterial endophytes for development into biological control agents for SDS, bacterial endophytes were isolated from the crown tissue and rhizomes of SDS resistant Midlawn and susceptible Tifgreen bermudagrass cultivars, including SDS infected and non-infected plants. Endophytic bacteria were putatively identified to genera by sequencing contigs of their 16S rDNA and BLAST matching these sequences to the NCBI database. This is the first report, to the best of my knowledge, of a *Geodermatophilus* sp. and an *Amycolatopsis* sp. as plant endophytes and the first observation of a *Chryseobacterium* sp. with *in vitro* antifungal attributes. In addition, a real-time PCR assay with TaqMan[®] chemistry was developed to detect absolute quantities *O. herpotricha* DNA in plant and soil samples from 8 SDS infected cultivars varying in resistance to SDS.

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REVIEW OF LITERATURE

INTRODUCTION

Microbial phytopathogens cause great damage to agricultural crops and threaten the world's supply of food, timber, and natural fiber products. Despite pathogen control methods, fungal diseases still cause billions of dollars worth of economic losses to agriculture each year. As a consequence of globalization, introductions of non-endemic organisms pathogenic to crops and native plants present challenges to disease control efforts. New solutions are needed for effective and environmentally-friendly control of plant diseases.

Efforts to control diseases in turfgrasses and agricultural crops face many challenges. Fungal plant pathogens are sometimes non-responsive to fungicides due to resistance. Furthermore, resistance in plants can be overcome by evolving or non-endemic plant pathogens. Details regarding the nature of plant-pathogen interactions have not been fully elucidated in all cases, nor has the role of non-pathogenic microbes in plant disease or disease resistance been elucidated.

Recent scientific breakthroughs have made it easier to study plant-pathogen interactions and to identify and measure abundances of pathogenic and non-pathogenic microbes. DNA technologies have allowed scientists to quantify gene expression, leading to a better understanding of plant responses to pathogens. Accumulation of DNA

sequences into public databases accelerate these discoveries. These modern techniques are now being applied to study turfgrass diseases.

Bermudagrass is an economically important turfgrass. Bermudagrass is used in recreational areas, athletic fields, and as a forage grass in the sunbelt of America and in Australia, New Zealand, Africa, and tropical and subtropical regions of the world. In fact, the cultivar Greg Norman-1 turf was the playing surface for American football's Super Bowl XXXIII in Miami and American baseball's 1999 World Series in Atlanta. Unfortunately, this bermudagrass cultivar is quite susceptible to a fungal pathogen that causes a disease known as spring dead spot (SDS).

The most devastating fungal pathogens of bermudagrass are the three *Ophiosphaerella* Spegazzini 1909 spp., *O. korrae* (J. C. Walker & A. M. Smith) Shoemaker & C. E. Babcock, *O. herpotricha* (Fr.:Fr.) J. C. Walker, and *O. narmari* Wetzel, Hulbert & Tisserat, all of which can cause SDS. Better control of SDS requires correct identification of the SDS pathogens, development of resistant bermudagrass cultivars, specific cultural practices and more consistent fungicide treatments when SDS outbreaks are observed. Major advances have been made in pathogen detection by Tisserat at Colorado State University and coworkers at Kansas State University and Martin and coworkers at Oklahoma State University (Tisserat et al. 1994; Wetzel III et al. 1996; Wetzel III et al. 1999a; Wetzel III et al. 1999b; Tisserat et al. 2004). Development of cultivars with improved disease resistance has been led by Taliaferro and coworkers at Oklahoma State University. Better understanding of the interactions of bermudagrass with pathogenic and non-pathogenic microbes should lead to improved control methods.

Description of Bermudagrass

Bermudagrass (*Cynodon* L. C. Richard) is a vigorous warm season perennial sod-forming turf and forage grass. Bermudagrass grows rapidly during optimal growth conditions, forming a lush, thick mat that is highly resistant to wear and recuperates rapidly from turf injuries (vehicle tire ruts, golf divots, wash-outs). *Cynodon* L. C. Richard belongs to the Family *Poaceae* and the Tribe *Cynodonteae* (Table 1). The genus *Cynodon* includes nine species, ten varieties, and numerous cultivars (Tables 2 and 3). The species *C. dactylon* (L.) Pers. was initially described by Carl von Linnaeus followed by Christiaan Hendrik Persoon (1761-1836) (Brummitt and Powell 1992). *C. dactylon* is native to India and eastern Africa (Braun 1967; Correll and Johnston 1970; Beard 1973; Duple 1996) and has a plethora of common names (Table 4). Bermudagrass was introduced into the United States of America (US) from India or Africa in the late 1700s and was considered one of the major grasses in the southern states by 1807 (Duple 1996; Deputy et al. 1998).

Table 1. The taxonomy of *C. dactylon* (L.) Pers. (Anonymous 1997a).

Kingdom *Plantae*
Division *Magnoliophyta*
Class *Angiospermae*
Subclass *Commelinidae*
Order *Cyperales*
Family *Poaceae* Barnhart
Tribe *Cynodonteae*
Genus *Cynodon* L. C. Richard
Species *Cynodon dactylon* (L.) Pers.

Table 2. The genus *Cynodon* L. C. Richard, (after de Wet and Harland 1970 and Harland et al. 1970 as cataloged by Taliaferro (Taliaferro 1995)).

<u>Epithet</u>	<u>Distribution</u>
<i>C. aethiopicus</i> Clayton et Harlan	East African rift valleys
<i>C. arcuatus</i> J. S. Presl. ex C. B. Presl.	Malagasy, southern India to northern Australia
<i>C. barberi</i> Rang. et Tad.	Southern India
<i>C. dactylon</i> (L.) Pers.	
var. <i>dactylon</i>	Cosmopolitan
var. <i>afghanicus</i> Harlan et de Wet	Afghanistan steeps
var. <i>aridus</i> Harlan et de Wet	Southern Africa northward to Palestine; east to South India
var. <i>coursii</i> (A. Camus) Harlan et de Wet	Madagascar
var. <i>elegans</i> Rendle	Southern Africa, south of lat. 13 °S
var. <i>polevansii</i> (Stent)	Near Barberspan, South Africa
<i>C. incompletus</i> Nees	
var. <i>incompletus</i>	South Africa; Transvaal to Cape
var. <i>hirsutus</i> (Stent) de Wet et Harlan	South Africa; Transvaal to Cape
<i>C. nlemfuensis</i> Vanderyst	
var. <i>nlemfuensis</i>	East Africa
var. <i>robustus</i> Clayton et Harlan	East Tropical Africa
<i>C. plectostachyus</i> (K. Schum.) Pilger	East Tropical Africa
<i>C. transvaalensis</i> Burt-Davy	South Africa
<i>C. x magennisii</i> Hurcombe	South Africa

Table 3. A list of bermudagrass cultivars released in the past 50 years. (Hanson 1972; Adams and Gibbs 1994; Alderson and Sharp 1994; Duble 1996; Deputy et al. 1998; Busey and Dudeck 2005).

<u>Name</u>	<u>Year Released</u>	<u>Developed by, Recommended Uses</u>
Coastal (Reg. No.1)	1943	Georgia Coastal Plain Experimental Station and Plant Science Research Division, ARS grazing and hay
U-3	1947	Released by ARS-USDA
Tiflawn	1952	Georgia AES
Midland (Reg. No. 2)	1953	Oklahoma AES, Georgia Coastal Plain Experimental Station, Plant Science Research Division, AES, pasture
Suwannee (Reg. No.6)	1953	Georgia Coastal Plain Experiment Station, Plant Science Research Division, ARS, grazing and hay
Tiffine	1953	Georgia AES, golf fairways, tees, before Tifgreen
Greenfield	1954	Oklahoma AES, pasture
Tifgreen	1956	Georgia AES, golf greens, fairways, tees

<u>Name</u>	<u>Year Released</u>	<u>Developed by, Recommended Uses</u>
Tiflawn (Reg. No. 4)	1956	Georgia Coastal Plain Experimental Station, Plant Science Research Division, AES, turf
Sunturf	1956	Alabama ASE
NK-37	1957	Northrup, King & Co.
Texturf	1957	Texas AES
Texturf 10	1957	Texas AES
Bayshore	1960	Florida AES
Royal Cape	1960	University of CA, Los Angeles, Plant Science Research Division, ARS., adapted to high salt areas of southern CA, turf
Tifway	1960	Georgia AES, golf courses, racetracks, lawns
Everglades	1962	Florida AES, putting green turf
Ormond	1962	Florida AES, golf tees and fairways
Tufcote	1962	SCS, National Plant Materials Center, Beltsville, MD, Maryland AES, heavy traffic areas, lawns, golf courses
Midway	1965	Kansas AES, turf
Tifdwarf	1965	Georgia AES, golf greens, superior putting quality, believed a vegetative mutant of Tifgreen
Santa Ana	1966	California AES, fine turf
Coastcross-1	1967	Georgia Coastal Plain Experimental Station and Plant Science Research Division, ARS grazing and hay
Pee Dee 102	1968	South Georgia AES, golf greens in the southeastern US, a vegetative mutant of Tifgreen
Midiron	1971	Kansas AES, turf
Hardie (Reg. No. 11)	1974	Oklahoma AES, pasture and hay production
McCaleb	1975	University of Florida Institute of Food and Agricultural Science, Agricultural Research and Educational Center, Ona, FL, perennial forage grass
Tifton 44 (Reg. No. 10)	1978	Georgia AES and Fr-SEA-USDA, grazing and hay
Ona	1979	University of Florida Institute of Food and Agricultural Science, Agricultural Research and Educational Center, Ona, FL, perennial pasture grass
Tifway II (Reg. No. 15)	1981	ARS, Georgia Coastal Plain Experimental Station, U. S. Golf Association Greens Section, U. S. Department of Energy, turf
Brazos	1982	Texas AES, ARS and Louisiana AES, pasture, hay
Guymon	1982	Oklahoma AES, general purpose
Tifton 68 (Reg. No. 14)	1984	ARS and Georgia AES, grazing and hay
Tifton 78 (Reg. No. 17)	1984	University of GA and ARS, grazing and hay
Vamont	1986	Virginia AES
NuMex Sahara	1987	New Mexico AES, general purpose turf

<u>Name</u>	<u>Year Released</u>	<u>Developed by, Recommended Uses</u>
C2	1988	D. Palmer Seed Co., Inc., turf in very alkaline soils
Florico	1988	University of Florida Institute of Food and Agricultural Science, Agricultural research and Educational Center, Ona, FL, USDA-ARS, TARS (Puerto Rico), perennial pasture grass
Florona	1988	University of Florida Institute of Food and Agricultural Science, Agricultural research and Educational Center, Ona, FL, perennial pasture grass
Tifton 10	1988	Georgia Coastal Plain AES and ARS, turf
Cheyenne	1989	Jacklin Seed Co. and Pennington Seed, turf and reclamation
Primavera	1989	Farmers Marketing Corp., general purpose turf
Midfield	1991	Kansas and Oklahoma AES, transition zone turf
Midlawn	1991	Kansas and Oklahoma AES, transition zone turf
Tifton 85	1991	USDA-ARS, coastal Plain Experimental Station, grazing and hay
Sonesta	1992	O. M. Scott & Sons Co., general purpose turf for golf courses
Sundevil	1992	Jacklin Seed Co., turf and reclamation
Quickstand	1993	Kentucky AES, heavy recreational use
GN-1	1995	Greg Norman Turf
Yukon	1996	Oklahoma ASE
MS-Choice	1996	Mississippi AES, lawns, sports fields, more shade tolerant
MS-Express	1996	Mississippi AES, golf putting greens, tennis greens
MS-Pride	1996	Mississippi AES, lawns, golf tees and fairways

Table 4. Common names of *C. dactylon*. The list is not all inclusive (Dastur 1950; Watt and Breyer-Brandwijk 1962; Ayensu 1981; Jayaweera 1981; Boulos 1983; Duke and Ayensu 1985; Oudhia 2001; Wu 2002).

<u>Language</u>	<u>Name</u>
Arabic	endjil, nihil, moddad, medjem, madjir, zabak, kexmir, tsil, raifa
Bengali	durba
Berber	tizmit, affer, agesmir, tagamait, imelzi, haffar, toungane, agouzinir
Chinese	tie xian cao (iron weed grass), gai ya gen (dog teeth), pa ti cao (crawling grass), ai shen cao (dwarf grass), bai mo da (bermudagrass)
French	gros chiendent, herbe du bermudes, chiendent pied de poule, chiendent d'Italic, dactyle, petit chiendent
Hindi	dhoboghas, dubra, durba, dub, huriyale, kabbar, kalighas, khabbal
Kanarese	garikihallu
Marathi	durva

<u>Language</u>	<u>Name</u>
Punjab	dhubkhabbal
Sanskrit	amari, bahuvirya, durmara, gauri, haritali, jaya, mahaushadhi, nahavari, niladurva, rhha, shasravirya, shadvala, shanbhavi, shaspha, shataparva, shitakumbhi, tiktapara, vamini, vijaya
Sotho	mohloa, mohlwa, hoholoa, morara, seihla
Tamil	arugampillu, hariali
Telug	garikagoddi
Tswana	mothowa, motwa
Xhosa	uqaqaqa
Zulu	isifulwane, ungwengwe, umqambalala, umqambalalane, uqethu

Additional common names are: Australian couch grass, Bahama grass, batawiesek week, Bermuda grass, Bermuakweekgras, Bermuda quick grass, Buffel grass, couch grass, creeping cynodon, creeping panic grass, devil's grass, dog tooth, elandskweek, fine couch grass, fine quack, fingers, Florida grass, fynkweekgras, gariesgras, germiston grass, hardekweek, Indian couch grass, Indiesekweek, kruisgras, kwaggakweek, kweekgras, lawn grass, oostindiesekweekgras, quagga quick, quick grass, regtewekgras, riverkweek, running grass, Scotch grass, scucch grass, star grass, vingergras, white quick grass, and wire grass.

Bermudagrass reproduces vegetatively from underground rhizomes and above ground stolons, and sexually by seed. Providing nutrients are not limiting, the grass is highly adapted to soils ranging from heavy clays to deep sands, acid, alkaline, and saline conditions. On the negative side, bermudagrass cannot withstand low temperatures, long periods of freezing, or even partial shade (Gould 1973; Turgeon 1991; Duble 1996).

The cultivars of bermudagrass include both natural and man-made hybrids. Bermudagrass cultivars, or 'improved' bermudagrasses, are found throughout the tropical and subtropical areas of the world. Improved bermudagrasses are heat and drought tolerant, moderately cold tolerant, and require high soil fertility for a healthy turf. Cultivars have been developed and released by Agricultural Experiment Stations (AES) of several US land grant institutions, the Crops Research Division-Agricultural Research Service-United States Division of Agriculture (ARS-USDA), Sod Growers Associations,

and other private interests (Adams and Gibbs 1994; Duple 1996; Deputy et al. 1998) (Table 3) for a variety of uses.

Uses and Occurrences of Bermudagrass

Bermudagrass is widely used as a turfgrass predominately for golf courses, polo fields, athletic playing fields, parks, other recreational areas, residential housing units, and roadside erosion and dust control (Duple 1996). In Oklahoma, bermudagrass turf is extensively used and the estimated replacement cost is approximately \$1.7 billion dollars.

Versatile bermudagrass is not only used for turf, forage, and pasture grass, but is an important component in ceremonies and an ingredient in folk remedies.

Bermudagrass is used in religious festivals in India and as an ingredient in herbal cures for diarrhea, scalp dryness, and headaches (Table 5).

Table 5. *Cynodon dactylon* is used for diverse medical ailments by many cultures (Dastur 1950; Watt and Breyer-Brandwijk 1962; Ayensu 1981; Cribb and Cribb 1981; Jayaweera 1981; Boulos 1983; Duke and Ayensu 1985).

Arrest bleeding	Ceylon, China, India, Pakistan, North Africa
Diuretic	Australia, Ceylon, China, India, North Africa, Pakistan, Philippines, West Indies
Dysentery	Ceylon, India, Pakistan
Epilepsy	Ceylon
Hysteria	Ceylon, India, Pakistan
Insanity	Ceylon, India, Pakistan
Inflammation of a body opening	Ceylon, India, Pakistan
Secondary syphilis	Ceylon, India, Pakistan
Gout	Australia, Madagascar, India, Pakistan
Rheumatic affections	Australia, Madagascar, India, Pakistan
Blood purifier	Africa, China, North Africa
Laxative	China
Urinary bladder inflammation	Australia, India, North Africa, Pakistan, West Indies

The exact qualities that make bermudagrass an excellent forage and turfgrass also make bermudagrass as an aggressive and invasive weed. The State of California has acknowledged the importance of bermudagrass as a weeds (*Cynodon* spp. and hybrids) and has placed them on the ‘noxious weeds’ list (California Department of Food and Agriculture 2005). A ‘noxious weed’ is “troublesome, aggressive, intrusive, detrimental, or destructive to agriculture, silviculture, or important native species, and difficult to control or eradicate” (California Department of Food and Agriculture 2005). Bermudagrass as a weed is an unwelcome visitor that usually requires much work to eradicate.

Pests and Diseases of Bermudagrass

Bermudagrass, though a versatile and extensively planted turfgrass, is vulnerable to a number of pests. Lucas and Bruneau (1995) wrote concerning bermudagrass, “Many pest problems . . . [diseases, weeds, insects, and animals] cause your turf to look bad ... If you are really unlucky, you may have all of them at one time.” Taliaferro (1995) listed 15 insects, 8 nematodes, and 17 fungi that are important pests of *Cynodon* spp. A list of these is described in Table 6.

Table 6. Important pests of bermudagrass including insects, bacteria, fungi and one miscellaneous pest. (Rogerson 1958; Shurtleff et al. 1987; Smiley et al. 1992; Sauer et al. 1993; Vargas 1994; Taliaferro 1995; Fermanian et al. 2003; Taliaferro et al. 2004).

Insects

Armyworm, *Spodoptera frugiperda* J. E. Smith

Bermudagrass mites, *Eriophyes cynodontiensis*

Bermudagrass scales, *Odonaspisruthae* spp.

Chinch bugs, *Bilssus leucopterus*

Grasshoppers, *Melanoplus* spp.

Ground pearls, *Margarodes* spp.

Phoenix billbug, *Sphenophorus phoeniciensis*

Insects

Pyralid grassworm, *Marasmia trapezalis* Guenée
Spittlebugs, *Prosapia bicincta* Say.
Sod webworm, *Fissicrambus haytiellus* Zinck.
Striped grass looper, *Mocis latipes* Guenée
Tawny mole cricket, *Scaptericus vicinus* Scudder
White grubs, *Phyllophaga* spp.

Nematodes

Awl, *Polichodorus* spp.
Burrowing, *Radopholus* spp.
Dagger, *Xiphinema* spp.
Lance, *Hoplolaimus* spp.
Lesion, *Pratylenchus* spp.
Needle, *Longidorus* spp.
Pin, *Paratylenchus* spp.
Root knot, *Meloidogyne* spp.
Spiral, *Helicotylenchus* spp.
Sting, *Belonolaimus* spp.

Bacteria

Bacteria wilt, *Xanthomonas campestris* pv. *graminis*

Fungi

Anthraxnose, *Colletotrichum graminicola* (Ces.) Wils.
Bermudagrass decline, *Gaeumannomyces graminis* (Sacc.) Arx & Oliver var. *graminis*
Brown patch, *Rhizoctonia* spp.
Brown stripe, *Cercosporidium graminis* (Fuckel) Deighton
Cercospora leaf spot, *Cercospora seminalis*
Copper spot, *Gloeocercospora sorghi* Bain & Edgerton ex. Deighton
Dollar spot, *Sclerotinia homoeocarpa* F. T. Bennett
Gray leaf spot, *Pyricularia grisea* (Cooke) Sacc.
Leaf blotch, crown, and root rot, *Bipolaris cynodontis* (Marig.) Shoemaker
Leaf blotch, *Drechslera cynodontis* Nelson, *Helminthosporium giganteum* Heald & Wolf,
H. rostratum Dreschl., *H. spiciferum* (Bain.) Nicot, *H. stenospilum* Dreschl., *H. triseptatum* Dreschl.
Leaf spot, *Exserohilum rostratum*
Leaf spot, leaf, crown, root rot, *B. sorokiniana*
Physoderma leaf spot, leaf streak, *Physoderma graminis*
Pink patch, *Limonomyces roseipellis* Stalpers & Loerckker
Powdery mildew, *Erysipha graminis* DC.
Pythium blight, grease spot, cottony blight, *Pythium aphanidermatum* (Edson) Fitzpatrick, *P. ultimum* Trow
Red thread, *Laetisaria fuciformis* (McAlp.) Burdsell
Rust, *Puccinia cynodontis* Lac. ex Desmaz
Spring dead spot (SDS), *Ophiosphaerella herpotricha*, *O. korrae* O. narmari

Fungi

Southern, Sclerotium blight, *Sclerotium rolfsii*

Stem, crown, and root necrosis, *B. spicifera*

Yellow leaf spot, *Drechslera tritici-repentis*

Yellow patch, Rhizoctonia yellow patch, *Rhizoctonia cerealis*

Zonate leaf spot, *D. gigantea*

Miscellaneous

Slime mold, *Physarum cinereum* (Batsch.) Pers.

Spring dead spot (SDS), caused by three *Ophiosphaerella* spp., is the single most destructive disease of bermudagrass (Tisserat et al. 1989, Doble 1996; Watschke et al. 1995; Wetzel III et al. 1999a) and is pathogenic to other grasses as well. The genus *Ophiosphaerella* was described by Spegazzini in 1909 and belongs to the Class *Ascomycetes* and the Order *Pleosporales* (Table 7). *O. korrae* causes necrotic ring spot in Kentucky (*Poa pratensis* L.) and annual bluegrasses (*P. annua*) and creeping red fescue (*Festuca rubra* var. *rubra*) (McCarty and Lucas 1989; Dernoeden 1999). Although the primary host of *O. herpotricha* is bermudagrass, *O. herpotricha* causes a patch disease in zoysiagrass (*Zoysia japonica* Steud.) and SDS in buffalograss (*Buchloe dactyloides*) (Green II et al. 1993; Dernoeden 1999).

The Fungal Genus *Ophiosphaerella* Spegazzini 1909

The taxonomy of *Ophiosphaerella* spp. has changed over the years, making older literature somewhat confusing. *Ophiosphaerella korrae* was first described in 1965 as *Ophiobolus herpotrichus* (Fr.) Sacc., redescribed in 1972 as *Leptosphaeria korrae* J. C. Walker & A. M. Smith, and finally renamed in 1989 as *Ophiosphaerella korrae*. *Ophiosphaerella narmari* was first described in 1972 as *Leptosphaeria narmari* J. C. Walker & A. M. Smith, then, in 1989 was reclassified as *Phaeosphaeria narmari* (J. C.

Walker & A. M. Smith) Shoemaker & C. E. Babcock, and, in 1999, redescribed as *Ophiosphaerella narmari* (Wetzel III et al. 1999a). In 1989, Tisserat et al. concluded *Ophiosphaerella herpotricha* (Fr.) Walker was a synonym for *Ophiobolus herpotrichus*. Landschoot (1993) assigned the following synonyms to *Ophiosphaerella herpotricha*, *Ophiobolus herpotrichus* (Fr.:Fr.) Sacc. & Roum., *Phaeosphaeria herpotricha* (Fr.:Fr.) L. Holm, *Ophiobolus medusae* Ellis & Everh. f. *brimi* Brenckle, *Ophiobolus oryzae* Miyabe, and *Scolecosporiella* sp. (anamorph).

Symptoms and Occurrence of Spring Dead Spot Disease

In the United States, SDS infects bermudagrass in locations where the plant goes into dormancy in the winter. The geographic zone of SDS is the at the northern range of bermudagrass adaptation (Tisserat 1989) where average temperatures in the late autumn are between 7.2 to 13.9 °C. The longer cold temperatures persist, the greater the disease (Fermanian et al. 2003).

The habit of SDS fungal pathogens is soilborne, ectotrophic (coating the exterior surface), and root-infecting (sending hyphae into the root) (Smiley and Fowler 1984). Optimum soil temperatures of 15 – 25 °C induce *O. herpotricha* colonization (Fermanian et al. 2003). The progression of the disease is slow, usually taking about 2 to 3 years to establish. The fungi first colonize the outer surfaces of underground structures forming an epiphytic dark coating of hyphae followed by hyphal penetration into the cortex to extract nutrients. SDS is most evident in three to six year old intensely managed bermudagrass turf. The disease becomes obvious when the grass breaks dormancy in the spring. Round, bleached areas, from three to one meter in diameter, indicate where SDS has killed the turf.

Table 7. The taxonomy of the genera *Ophiosphaerella* (Landschoot 1993).

Kingdom *Fungi*
Division *Ascomycota*
Subdivision *Ascomycotina*
Class *Ascomycetes*
Subclass *Loculoascomycetidae* (*Loculoascomycetes*)
Order *Pleosporales* Luttrell ex Barr 1983
Family *Phaeosphaeriaceae* M. E. Berg 1979
Genus *Ophiosphaerella* Spegazzini 1909
Species *Ophiosphaerella herpotricha* (Fr.:Fr.) J. C. Walker
Species *Ophiosphaerella korrae* (J. C. Walker & A. M. Smith)
Shoemaker & C. E. Babcock
Species *Ophiosphaerella narmari* Wetzel, Hulbert & Tisserat,
comb. nov.

The recent history of SDS spans two continents. Symptoms similar to SDS have been observed sporadically in Oklahoma since 1936, and in 1960 the term spring dead spot was coined by Wadsworth and Young. SDS was first documented in Australia in 1965, and shortly thereafter in New Zealand. SDS was first reported in North Carolina in the late 1960s (Smiley 1993) and first documented in southern California in 1983 (Endo et al. 1985). During these years, identifications of causal agents were tentative, incorrect, or unknown because they relied on symptoms or fungal morphological assessments that often gave ambiguous conclusions. With the advent of molecular techniques, identification of *Ophiosphaerella* spp. can now be made with certainty (O'Gorman et al. 1994; Tisserat et al. 1994; Wetzel III et al. 1999a; Wetzel III et al. 1999b).

Identification of the pathogen in a host is of the utmost importance for successful disease control. Tisserat et al. (1994) developed species-specific DNA probes derived from internal transcribed spacer (ITS) regions from *O. korrae* and *O. herpotricha*. The

DNA probes were used to identify these fungi in artificial and naturally infected bermudagrass roots. The *O. herpotricha* DNA probe amplified DNA from *O. herpotricha* but not 30 other isolates, including *O. korrae*. The *O. korrae* DNA probe detected only *O. korrae* and not the other 30 isolates tested. Wetzell III et al. (1999a) were able to identify, for the first time, the presence of *O. narmari* Wetzell, Hulbert & Tisserat, comb nov. (= *Leptosphaeria narmari*) in North America using species-specific DNA probes. With these and other molecular and microbiological tools, identification of SDS pathogens has become more accurate. The markers assist researchers in defining their geographical range. SDS has been found to be widespread and caused by different species in different regions (Table 8).

Table 8. Spring dead spot fungi and documented locations (Endo et al. 1985; Tisserat et al. 1989; Jackson 1993; Venkatasubbaiah et al. 1994; Chastagner and Hammer 1997).

<u>Species</u>	<u>Location</u>
<i>Ophiosphaerella herpotricha</i>	Kansas, Kentucky, Louisiana, Missouri, North Carolina, Oklahoma, Texas
<i>O. korrae</i>	California, Colorado, Maryland, Michigan, New York, Utah, Washington, Australia
<i>O. narmari</i>	USA, Australia, New Zealand

Options for Control of Spring Dead Spot Disease

The most common approach to control of fungal diseases involves the use of chemical fungicides. Fungicide treatments recommended for use in Alabama, Kentucky, Maryland, and Texas were ineffective in controlling SDS in Kansas and Oklahoma (Anonymous 1999a; Vincelli 2000; Dernoeden 2000; Hagan 1997). Fungicide treatments discouraged for the control of SDS by the Oklahoma and Kansas Cooperative Extension

Services who recommended specific cultivation practices to contain and control SDS (Martin and Hudgins 1998; Tisserat 1998) (Table 9). Extensive cultivation practices are time consuming, expensive, and only partially effective, and need to be evaluated over several years. Furthermore, there are no truly resistant varieties of bermudagrass although efforts to develop these are in progress. Several of these control practices may be cost effective for home lawns or small turfgrass plots in other geographic areas but can be cost prohibitive for larger areas, such as: golf courses, parks, and athletic fields.

Table 9. Cultivation practices recommended by the Oklahoma, Kansas, and Alabama Cooperative Extension Services to reduce the impact of SDS on bermudagrass (Hagan 1997; Martin and Hudgins 1998; Vincelli and Powell 2000).

- Removal of excess thatch
- Maintenance of soil pH at 5.8 – 6.2
- Light annual liming
- Annual core aerification
- Soil testing for potassium and phosphorus yearly and add if deficient
- Use ammonium sulfate or ammonium chloride for nitrogen application, apply lightly but frequently through the growing season
- Monthly micronutrient sprays
- Use slow-release forms of organic or inorganic fertilizers
- Autumn potash application
- No N, P, and K applications after the first week in September

The use of fungicides is not only costly but potentially toxic to nontarget organisms, including fungicide applicators. Six fungicides are recommended to control SDS (Table 10) but these fungicides pose risks to ecosystems and human health. At low levels, fenarimol and thiophanate-methyl are toxic to fish (Anonymous 1998, 2000a, b) with the remaining four fungicides toxic to freshwater, estuarine, and marine fish and invertebrates (Anonymous 1999b, 2000c, d, e). Azoxystrobin, fenarimol, propiconazole,

and thiophanate methyl are toxic to the liver, while fenarimol shows reproductive and fertility effects, and of myclobutanil and thiophanate-methyl shows reproductive and embryoteratotoxicity (Anonymous 1997b, c, 1987, 1999b, 2000a-d). These fungicides can be transported out of treated areas toward nontarget areas by winds and water runoff, posing a threat to surrounding and distant ecosystems. Furthermore fungicides may lose their effectiveness over time if fungal pathogens develop resistance (Clarke et al. 1997). These ramifications demonstrate the need to develop effective and safer alternatives to control SDS.

Table 10. Fungicides recommended for use against spring dead spot.

<u>Common Name</u>	<u>Trade Name</u>
Azoxystrobin	Heritage, Abound
Chlorothalonil	Daconil, Bravo
Fenarimol	Rubigan
Myclobutanil	Eagle
Propiconazole	Banner Maxx, Tilt
Thiophanate-methyl	Fungo, 3336WP

(Hagan 1997; Martin 1999; Vincelli and Powell 2000; Anonymous 2001).

SDS remains a problem even when the most resistant bermudagrass cultivars and most prudent cultivation practices are used. Given the limited effectiveness of fungicide treatments, biological control agents hold promise as an more environmentally friendly and time saving alternative. A safe and cost-effective alternative to controlling plant diseases is through the use of antagonistic organisms in a process known as biological control. Natural bacteria, as opposed to transgenic bacteria, are perhaps the most promising agents for biological control of fungal diseases in plants. In agriculture,

biological control is a widely accepted strategy for controlling pests, and presents a reasonable and less expensive alternative to massive fungicide treatments or extensive cultivation practices (Table 11).

Table 11. A small sampling of commercial biological control agents against soilborne crop diseases (Hinton and Bacon 1995; Anonymous 2000f; Vasudevan et al. 2002; Ritter 2003).

<u>Biological Control Organism</u>	<u>Target</u>	<u>Crop</u>
<i>Agrobacterium radiobacter</i>	<i>Agrobacterium tumefaciens</i>	Trees
<i>Bacillus laterosporus</i> (V)	<i>Rhizoctonia solani</i>	Rice
<i>Bacillus pumilus</i> (V)	<i>Rhizoctonia solani</i>	Rice
	<i>Sclerotium oryzae</i>	Rice
<i>Bacillus subtilis</i> GB03	<i>Rhizoctonia</i>	Horticultural
	<i>Pythium</i>	Turfgrass
	<i>Fusarium</i>	
	<i>Phytophthora</i>	
<i>Bacillus subtilis</i> B2g (M)	<i>Pythium ultimum</i>	
	<i>Rhizoctonia solani</i>	
<i>Bacillus subtilis</i> QST-713 (r)	Fungi	Tomato, lettuce, grapes
<i>Burkholderia cepacia</i>	<i>Pseudomonas cepacia</i>	Legumes
	<i>Rhizoctonia</i>	Wheat
	<i>Phthium</i>	Barley
	<i>Fusarium</i>	Cotton
		Grain Sorghum
		Vegetable corps
<i>Enterobacter cloacae</i> (H B)	Fungi	Fruits, vegetables
<i>Pseudomonas aeruginosa</i> (V)	<i>Drechslera oryzae</i>	Rice
<i>Pseudomonas aureofaciens</i> Tx-1	Dollar spot	Turfgrass
	Anthraxnose	
	<i>Pythium aphanidermatum</i>	
	Michrochium patch (pink snow mold)	
<i>Pseudomonas fluorescens</i> A506	Frost damage	Almond
	<i>Erwinia amylovora</i>	Fruit trees
	Russet-inducing bacteria	Tomato
<i>Pseudomonas fluorescens</i> (V)	<i>Magnaporthe grisea</i>	Rice
<i>Pseudomonas putida</i> (V)	<i>Rhizoctonia solani</i>	Rice

<u>Biological Control Organism</u>	<u>Target</u>	<u>Crop</u>
<i>Serratia marcescens</i> (V)	<i>Rhizoctonia solani</i>	Rice
<i>Stenotrophomonas maltophilia</i> C3	<i>Bipolaris sorokiniana</i>	Turfgrass (Z)
<i>Streptomyces griseoviridis</i> K61	<i>Fusarium</i> spp.	Ornamental
	<i>Pythium</i> spp.	Vegetable crops
	<i>Phytophthora</i> spp.	

Endophytic Bacteria

Before 1876, bacteria were thought to appear by spontaneous generation. In spite of Pasteur and Koch's discovery in 1876 that mammalian anthrax was caused by a bacterium, and T. J. Burril's findings in 1878, that fire blight disease of pomes was also caused by a bacterium, scientists were skeptical until the 20th century that bacteria could be found in plants. Studies from 1876 to 1896 supported the hypothesis that healthy plants did not contain bacteria, and the subject was largely ignored from 1896 to 1948, when fewer than 25 scientific papers dealt with plants and bacteria (Hollis 1951). Interest re-emerged in the mid 1950s, when studies of the biology and ecology of bacteria in plant roots began. Philipson and Blair (1957), documenting the mixed bacterial flora of clover roots, isolated three groups of endophytic bacteria: *Aerobacter cloacae*, *Bacillus megatherium*, and *Flavobacterium rhenanus*.

The definition of the term endophyte (Greek, 'endon' within, 'phyte' plant) has undergone several modifications. The original definition of endophyte was used to describe fungi living inside plants without causing disease (Chanway 1996). Then the term endophyte was expanded to include parasites (biotrophic parasites to facultative saprotrophic), mutualists (biotrophic mutualists, benign commensals to nectotrophic), and antagonistic pathogens (Stone et al. 2000). Currently, and in this study, the definition of

endophyte includes only microorganisms that reside inside a plant, during part or all of their life cycle, without causing disease symptoms (Chanway 1996).

Microbial endophytes have a sustained intimate relationship with plants. They live in virtually all plant tissues (Table 12); some are benign, some enhance plant growth, and some impact disease severity. The endophyte-plant relationship may have begun when plants first evolved on earth. Some specimens of fossilized plant tissue show plant-microbe associations (Strobel 2003). During the past 50 years, a small fraction of the approximately 300,000 plant species on earth have been subjects for endophyte studies, and all of these plants, several hundred, host a complement of microbes (Strobel 2003). Bacterial endophytes are closely associated with the plant, and some also thrive in the rhizosphere. Seed endophytes are usually passed into the germinating plant and are thereby passed on from generation to generation through a process of vertical transmission. Some soil bacteria have the potential to either penetrate or enter the roots through wounds and travel into the shoot system.

Bacteria gain entry to plants through a variety of ways, including natural openings, such as hydathodes, lenticles, micropores, and stomates; natural wounds, such as leaf and bud scale scars; and wounds caused by external forces, such as wind or pathogens. Roots may be the preferred site of entry for bacterial endophytes (Hallmann 2001). Bacteria enter the root from ruptures in the epidermis made by emerging roots, at the junction of a root hair and its epidermal cell, and between epidermal cells (Parke 1991). Hydrolytic enzymes that are capable of hydrolyzing the plant cell wall may be secreted by bacterial endophytes and may provide *the mechanisms* for endophytic entry into plant roots (Quadt-Hallmann et al. 1997; Kovtunovych et al. 1999).

Table 12. Bacterial endophytes isolated from an assortment of plants and plant organs. For a more exhaustive list, see Appendices 7, 8 and 10.

Endophyte	Plant	Tissue	Reference
<i>Acetobacter</i>	Pineapple	Plant tissues	(Tapia-Hernandez et al. 2000)
<i>Acetobacter</i>	Sugarcane	Stem	(Dong et al. 1994)
<i>Acetobacterium</i>	Sea Grass	Cortex	(Kusel et al. 1999)
<i>Achromobacter</i>	Citrus	Xylem	(Gardner et al. 1982)
<i>Acidovorax</i>	Clover	Roots	(Sturz et al. 1998)
<i>Aerococcus</i>	Cotton	Plant tissues	(Chen et al. 1995)
<i>Agrobacterium</i>	Carrot	Plant tissues	(Surette et al. 2003)
<i>Agrobacterium</i>	Healthy rose	Plant tissues	(Marti et al. 1999)
<i>Arthrobacter</i>	Canola	Root	(Germida et al. 1998)
<i>Azoarcus</i>	Grass	Plant tissues	(Hurek et al. 2002)
<i>Azospirillum</i>	Rice	Root	(Engelhard et al. 2000)
<i>Bacillus</i>	Aspen	Wood	(Knutson 1973)
<i>Bacillus</i>	Corn	Kernel	(Bacon and Hinton 2002)
<i>Bacillus</i>	Live oak	Plant tissues	(Brooks et al. 1994)
<i>Bacillus</i>	Wheat	Root	(Germida and Siciliano 2001)
<i>Burkholderia</i>	Banana	Plant tissues	(Pan et al. 1997)
<i>Burkholderia</i>	Rice	Root	(Englehard et al. 2000)
<i>Clavibacter</i>	Grapevine	Xylem	(Bell et al. 1995)
<i>Clostridium carbonei</i>	Pinto Beans	Plant tissues	(Thomas Jr. and Graham 1948)
<i>Corynebacterium</i>	Sugar beet	Root	(Jacobs et al. 1985)
<i>Curtobacterium</i>	Yam	Tuber	(Mantell 1998)
<i>Enterobacter</i>	Spinach	Root	(Tsuda et al. 2001)
<i>Enterobacter</i>	Lemon	Root	(Gardner et al. 1982)
<i>Erwinia</i>	Aspen tree	Wood	(Knutson 1973)
<i>Gluconacetobacter</i>	Sugarcane	Plant tissues	(Boddey et al. 2003)
<i>Herbaspirillum</i>	Rice	Root	(Englehard et al. 2000)
<i>Methylobacterium</i>	Scots pine	Plant tissues	(Mattila 2001)
<i>Mycobacterium</i>	Scots pine	Branch Bud	(Mattila 2001)
<i>Nocardia</i>	Citrus	Branch	(Araújo et al. 2002)
<i>Pantoea</i>	Potato	Tuber	(Sturz et al. 1999)
<i>Proteus</i>	27 plants	Ovule,seed	(Mundt and Hinkle 1976)
<i>Pseudomonas</i>	Alfalfa	Root	(Gagné et al. 1987)
<i>Pseudomonas</i>	Elm	Stem, root	(Mocali et al. 2003)
<i>Pseudomonas</i>	Live oak	Plant tissues	(Brooks et al. 1994)

Endophyte	Plant	Tissue	Reference
<i>Pseudomonas</i>	Sorghum	Stem	(Zinniel et al. 2002)
<i>Stenotrophomonas</i>	Elm	Stem, root	(Mocali et al. 2003)
<i>Streptomyces</i>	Laurel	Plant tissues	(Nishimura et al. 2002)
<i>Microbacterium</i>	Trufgrass	Seed, root	(Sundaram et al. 1988)
<i>Xanthomonas</i>	Mulberry	Shoot	(Sato et al. 2000)

Bacterial endophyte studies, since the early 1980s, concentrated mainly on documenting genera, ecological and population dynamics, growth-promoting endophytes, and antibiosis towards known pathogens. Bacterial endophyte studies have also included comparisons among different cultivars and between healthy and diseased plants.

Fundamental ecological studies of endophytic bacterial population dynamics in corn and cotton roots were conducted by McInroy and Kloepper (McInroy and Kloepper 1995), who found that corn stems and roots were colonized with endophytic bacteria at the time of seedling emergence in the field. The number of colony forming units (CFU) present in surface sterilized corn and cotton seeds planted in non-sterilized potting mix were three-fold higher in corn than cotton six days after planting. The seed endophytic bacterial population dynamics in cotton petioles and bolls were established one year after planting in the field.

The taxonomic diversity and abundance of endophytic bacteria may be influenced by plant cultivar. Sturz and Christie (1998), characterizing the culturable bacterial endophytes (Table 12) from the roots of four red clover (*Trifolium pratense* L.) cultivars found differences in species richness and abundance between cultivars. In contrast, Sturz et al. (1999) found no significant differences in species richness or abundance when he characterized culturable bacterial endophytes from the tubers of four cultivars of potato. In a related study, Adams and Kloepper (2002) characterized nine cotton (*Gossypium*

hirsutum L.) cultivars ranging in susceptibility to Fusarium wilt. Cultivar differences were found in the total endophytic bacterial population of four day old radicles.

Microbial abundances did not correspond with the susceptibility of the cultivars.

Germida and Siciliano (2001) documented a higher species diversity among culturable bacterial endophytes in the roots of recent cultivars of rice as compared to that in an ancient land race.

Bacterial endophytes not only colonize the cortex of roots and stems but can enter the stele and the xylem. When Gagne et al. (1987) investigated the populations in root and crown xylem tissue of healthy field-grown alfalfa plants, 6.0×10^3 to 4.3×10^4 colony forming units (CFUs) per g of fresh xylem sap, were measured. Age, cultivar, or sampling site did not affect endophytic bacterial populations. Gardner et al. (1982) studied the bacterial endophyte population in the xylem of Florida citrus trees. They vacuum extracted xylem fluid from healthy and young diseased trees (tree decline disease, no pathogen named). Their data suggest average bacterial counts from the years 1979 through 1981 were consistently higher in the diseased trees than in the healthy trees. They concluded xylem bacteria played an “important role in the physiology of citrus.”

Disease agents in plants may influence the diversity and abundance of bacterial endophytes because of competition and antibiosis, which may either be increased or decreased depending upon the pathogen and bacterial endophytes. Araujo et al. (2002) found the branches of citrus trees infected with *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis, had greater bacterial endophyte diversity than non-infected citrus plants. Also, potato plants infected with *Erwinia carotovora* subsp. *atroseptica* had a higher bacterial endophytic diversity than non-infected plants (Reiter et al. 2002).

Nitrogen fixing bacteria, previously thought to colonize only legumes, have been documented in non-legume plants. Baldani et al. (1986) characterized a previously undescribed nitrogen fixing root endophytic genus, *Herbaspirillum seropedicae* gen. nov., sp. nov. isolated from corn, sorghum, and rice. Barraquio et al. (1997) also isolated nitrogen fixing bacterial root endophytes from rice to investigate the colonization, persistence, nitrogen fixation, and unique combinations of endophytic nitrogen fixing bacteria. They noticed higher nitrogen fixing bacterial populations in roots during the grain ripening stage in field-grown IR72 rice plants without nitrogen fertilizer. Stoltzfus et al. (1997) wanted to increase the growth rate and yield of rice through naturally occurring endophytic nitrogen fixing bacteria isolated from rice roots, or an endophytic bacterium that could be genetically engineered to fix nitrogen. They were successful in isolating 24 nitrogen fixing bacterial species from rice roots. Along with the beneficial aspect of nitrogen fixing, some bacterial endophytes also displayed anti-pathogenic properties. These, and other discoveries, enticed more scientists to investigate endophytes in different plant genera.

The bacterial colonization of external lodgepole pine seedling roots and the influence upon the seedlings were studied by Shishido et al. (1995). Greenhouse grown pine seedlings assayed nine weeks after inoculation with the endophyte *Bacillus polymyxa* Pw-2 grew “significantly taller” with “significantly” more shoot and root biomass than non-inoculated seedlings. Bacterial endophytes have been documented in different root tissues and found to be, in some cases, not just benign but beneficial.

Bacterial endophytes have been and are currently investigated for antibiotic properties. In 1995, Hinton and Bacon found *Enterobacter cloacae* to be antagonistic

against the corn pathogen *Fusarium moniliforme* and two other mycotoxin producing fungi. Adhikari et al. (2001) challenged *in vitro* the rice pathogenic fungi *Achyla klebsiana* and *Pythium spinosum* with 3 *Pseudomonas* spp. and *Sphingomonas trueperi* and showed that all inhibited fungal growth. Sturz et al. (1999) isolated 13 endophytic bacteria from potato tubers that were antagonistic against *Fusarium* spp. The grass bacterial endophyte *Stenotrophomonas maltophilia* strain C3, field tested on tall fescue cv. 'Kentucky 31', controlled *Rhizoctonia solani* Kuhn, the causal agent of brown patch disease, on one of six field plots (Giesler and Yuen 1998). *S. maltophilia* also inhibited *R. solani* and *Verticillium dahliae* var. *longisporum* (Berg et al. 1996).

Bacterial Endophytes as Biological Control Agents

There are numerous advantages in using endophytic bacteria as biological control agents. Some endophytic bacteria survive in the surrounding plant rhizosphere, readily enter and colonize the host (Kageyama et al. 1992; Pleban et al. 1995; Tsuda et al. 2001), and retain their effectiveness through storage (Fravel 2000; Ritter 2003). Some endophytic bacteria excrete chitinases and proteases, produce secondary metabolites with antibiotic properties, and induce resistance in plants (Table 13). Endophytic bacteria can be as effective as fungicides in controlling select fungal pathogens, minimizing environmental threats and negative impacts on human health.

Table 13. Bacterial endophytes with antagonism towards phytopathogenic fungi. For a more exhaustive list, see Appendix 10.

Bacterial Endophyte	Isolated From	Antagonism Property	Host Plant or <i>in vitro</i>	Pathogen	Reference
<i>Agrobacterium tumefaciens</i>	Potato tuber	Antagonism	<i>in vitro</i>	<i>Phytophthora infestans</i> A1, A2	(Sturz et al. 1999)
<i>Bacillus cereus</i>	<i>Sinapis</i>	Chitinase	<i>in vitro</i>	<i>Rhizoctonia solani</i>	(Pleban et al. 1995)
<i>B. licheniformis</i>	Oilseed rape	Protease	<i>in vitro</i>	<i>Verticillium longisporum</i>	(Graner et al. 2003)
<i>B. mojavensis</i>	Corn kernel	Antagonism	<i>in vitro</i>	<i>Fusarium moniliforme</i>	(Bacon and Hinton 2002)
<i>B. pumilus</i>	Endophyte	Induced defense-related ultrastructural modifications	Pea	<i>Fusarium oxysporum</i> f. sp. <i>pisi</i>	(Benhamou et al. 1996)
<i>Burkholderia (Pseudomonas) cepacia</i>	Asparagus	Mycelia deformation	Banana	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> race 4	(Pan et al. 1997)
<i>Cytophaga johnsonae</i>	Oilseed rape	Protease	<i>in vitro</i>	<i>Verticillium longisporum</i>	(Graner et al. 2003)
<i>Paenibacillus polymyxa</i>	Oilseed rape	Protease	<i>in vitro</i>	<i>Verticillium longisporum</i>	(Graner et al. 2003)
<i>Serratia plymuthica</i>	Endophyte	Induced Resistance	Cucumber	<i>Pythium ultimum</i>	(Benhamou et al. 2000)
<i>Streptomyces</i> sp.	Perennial ryegrass	1-N-methylalbono-ursin	<i>in vitro</i>	Not stated	(Gurney and Mantle 1993)
<i>Streptomyces</i> sp.	Mountain Laurel	Antibiotic	Laurel	<i>Pestalotiopsis sydowiana</i>	(Nishimura et al. 2002)

In most conventional biocontrol protocols, the agent is applied directly to the soil. Some biocontrol bacteria are capable of establishing populations in the soil (Reinhold-Hurek et al. 1986; McInroy and Kloepper 1995; Germida et al. 1998; Germida and Siciliano 2001), while others cannot compete with indigenous strains. Those that survive and proliferate may enter the root system at points where secondary roots emerge,

through the root epidermis near root hairs and wounds (Agarwal and Shende 1987; Gagné et al. 1987; Kobayashi and Palumbo 2000). Bacterial species able to enter the plant and flourish within the apoplastic space can establish populations within the plant and theoretically reduce the development of pathogen-caused disease (Benhamou et al. 2000; Chen et al. 2000; Tsuda et al. 2001). Bacterial endophytes, once inside a plant, can move from the site of entry to other plant parts (Hall et al. 1986; Marti et al. 1999). The way in which these endophytes move around in the plant is unknown but may relate to the use of cell wall degrading enzymes (Pleban et al. 1995; Pan et al. 1997).

The bacterial endophyte may exert direct or indirect effects on the growth and establishment of plant pathogens. Bacterial endophytes can produce and secrete extracellular substances that provide direct control over the growth and reproduction of phytopathogens. Some endophytes produce chitinases that dissolve the cell wall of pathogenic fungi (Pan et al. 1997) and cell wall digestive proteases (Pleban et al. 1995; Pan et al. 1997; Graner et al. 2003). Other endophytes can produce secondary metabolites, such as 1-N-methylalbonoursin (Gurney and Mantle 1993) and munumbicins A-D (Castillo et al. 2002), both produced by *Streptomyces* sp. with antibiotic properties. However, most *in vitro* and *in planta* studies documenting antagonism by bacterial endophytes have not determined the mode of protection (Brooks et al. 1994; Chen et al. 1995; Adhikari et al. 2001; Coombs et al. 2003).

A number of bacterial endophytes display *in vitro* and *in vivo* antibiotic activity and a few control fungal plant disease as well as commercial fungicides.

Stenotrophomonas maltophilia produces an extracellular serine protease that protects sugar beets against *Pythium*-mediated damping-off disease at a rate equivalent to that by

chemical fungicides (Dunne et al. 2000). *Pseudomonas aureofaciens* TX-1 produces and secretes phenazine-1 carboxylic acid, and was as effective at controlling the dollar spot fungus (*Sclerotinia homeocarpa*), now *Lanzia* and *Moellerodiscus* spp., on creeping bentgrass (*Agrostis stolonifera* L. var. *palustris* [Huds.]) as the fungicides triadimefon and chlorothalonil (Powell et al. 2000). Extracellular chitin produced by *S. maltophilia* C3 controlled *Uromyces appendiculatus*, the causal agent of bean rust, to a degree comparable to that by thiophanate methyl or thiophanate methyl combined with manganese ethylenebisdithiocarbamate (Yuen 2001). In growth chamber studies, *P. aureofaciens* controlled dollar spot as well as did propiconazol but not as effectively as did azoxystrobin. *Enterobacter cloacae* controlled Pythium foliar blight on 7 to 10 week old ryegrass (*Lolium* L. spp.) plants comparable to iprodione and propiconazole (Uddin and Viji 2002). Not only can *S. maltophilia*, *P. aureofaciens*, and *E. cloacae* thrive as endophytes, they are also rhizosphere competent, making them, and other bacterial endophytes with similar attributes, promising biological control agents.

Additional advantages of using bacterial endophytes for biological control include the induction of a localized resistance response by some (Benhamou et al. 2000) and defense-enhancing ultrastructural modifications by others (Benhamou et al. 1996; M'Piga et al. 1997) in plants. In addition to the antifungal properties of endophytes, some endophytes stimulate plant growth (Gardner et al. 1984; Nejad and Johnson 2000; Barka et al. 2002). For example, seven bacterial endophyte species isolated from clover roots and potato tubers promoted plant growth in potato and displayed *in vitro* antagonism towards *Rhizoctonia solani* (Sturz et al. 1998). The mechanisms of plant growth enhancement were not determined in these studies, but may have to do with the control of

the disease pathogen itself or the production of hormonal substances that increase plant growth rates.

The optimal biological control organism will increase plant biomass and yield, protect the host against disease at the site of infection, and induce disease resistance throughout the plant. Systemic resistance can be elicited by lipopolysaccharides from the outer cell wall of Gram-negative bacteria (Newman et al. 1995; Coventry and Dubery 2001). The elicitation produces signaling compounds such as salicylic acid, jasmonic acid, and ethylene (Van Loon and van Strien 1999). These substances trigger a complex signaling cascade that increases expression of pathogenesis-related proteins including chitinases and proteases. This altered gene expression has morphological and biochemical consequences, including production of secondary metabolites such as siderophores (Becker and Cook 1988; Loper 1988), coenzymes (Palva et al. 1993), cyanic acid, and several antibiotics (Ahl et al. 1986; Duffy and Defago 1999). The complete range of mechanisms and signaling pathways involved in systemic resistance have not been fully determined and remain an active area of research that promises to aid development of biological control agents.

There are precedents for use of bacteria as successful biological control agents for plant diseases and include the commercial products: Companion (*B. subtilis* (Ehrenberg 1835) Cohn 1872 strain GB03, Growth Products, White Plains, NY), Subtilex (*B. subtilis*, The MicroBio Group Ltd., Boulder, CO), Spot-Less (*P. aureofaciens* Kluyver 1956 strain TX-1, Eco Soil Systems, Inc., San Diego, CA) (Fravel 2000), and Serenade (*B. subtilis* QST-713, AgraQuest Inc., Davis, CA) (Ritter 2003) all possess a broad range fungicidal activity.

Even with the advantages of using bacterial endophytes as biological control agents, there are some challenges to meet. Bacterial endophytes possessing antibiotic properties can display different levels of antagonism on different nutrient media, suggesting that the nutritional environment is important for the expression of antagonism (James and Gutterson 1986; Milner et al. 1996; Duffy and Defago 1999).

The majority of initial studies assessing the antibiosis of endophytic bacteria are executed under strictly controlled conditions in the laboratory and or greenhouse (Pleban et al. 1995; Nejad and Johnson 2000; Tsuda et al. 2001; Coombs et al. 2003) but corresponding activity may be absent under field conditions. The reasons for this may be several fold. The field environment is typically more complex and diverse than that found in the laboratory or even the greenhouse. Laboratory cultured bacteria in field conditions may fail to compete with the resident bacteria. The variable and diverse environment in the field may not support the expression of the antimicrobial activity (Sivan and Chet 1992; Deacon and Berry 1993; Bacon and Hinton 2002; Handelsman 2002). Although a few biocontrol agents have performed successfully in field trials (Gnanamanickam and Mew 1992; Raupach and Kloepper 1998; Dunne et al. 2000) there remain many obstacles to the successful development of a biocontrol agent.

There is an enormous untapped pool of endophytic bacteria with promise as biological control agents. Important considerations for developing endophytes as biological control agents include: maintaining high populations of specific pathogen acting endophytes in plant tissues, assuring that antagonistic properties of endophytes are expressed in the plant, optimizing or directing the colonization of endophytes to specific tissues, promoting the long term survival of the endophyte in the plant tissues, promoting

the expression of antifungal properties at levels sufficient for effective disease control. Additional difficulties in using endophytic species include the development of a large scale field-based application procedure. These represent important aspects for future research.

Molecular Characterization of Bacteria in Plants and Soils

The identification of bacterial endophytes is critical to the discovery and development of microbial biocontrol agents. The three methods most relied upon are morphology assessments, biochemical assays, and DNA sequence analysis. Other protocols discriminate bacteria based on analyses of the fatty acid composition or the G+C mol % of the genomic DNA. Some protocols apply to specific groups of bacteria, such as comparing sequences of the nitrogenase enzyme (*nif*HDK) of nitrogen-fixing microorganisms (Watson 1994).

The traditional and most straightforward method for bacterial identification is documenting morphological traits coupled with biochemical assays. Biochemical tests used to classify bacteria include, but are not limited to, the Gram stain reaction, aerobic or anaerobic growth, pH and temperature limits for minimum and maximum growth rates, and various nutritional requirements or responses to stress. A multiplexed approach to biochemical assays is provided by the Biolog GN MicroPlate coupled with the Biolog GN computer database (Biolog, Inc. Hayward, CA).

Morphological and biochemical traits can be plastic and are not as reliable for identifying organisms as information in the genetic code. Several popular approaches are based upon comparisons of DNA fragment lengths using gel electrophoresis. Genomic DNA fingerprints are often used to identify bacteria because they provide a high level of

taxonomic identification. Restriction fragment length polymorphism (RFLP) uses restriction enzymes to cut genomic DNA into fragments that are separated by gel electrophoresis to produce the DNA fingerprint. Classifications are made based on similarity of electrophoretic patterns to those from reference organisms.

Another DNA fingerprinting protocol called rep-polymerase chain reaction (rep-PCR) involves the PCR amplification of genomic DNA fragments using primers from repetitive sequences. Short repetitive DNA sequences are highly conserved, distributed sporadically throughout the bacterial genomic DNA, and can be specific to the strain level (Versalovic et al. 2004; de Bruijn 1992). Amplification of genomic DNA is initiated at one rep-PCR primer and is terminated at the next annealed primer, yielding fragments of different lengths. These fragments of genomic DNA are separated by gel electrophoresis to produce a DNA fingerprint. In the same manner as RFLP, the fingerprints are matched to a known organism and classification is based on pattern similarities. The drawbacks of genomic fingerprinting are: (1) an expensive computer program is needed for pattern comparisons, (2) these techniques are unable to resolve nucleotide sequence differences in fragments of similar length (3) there is an absolute need for reference organisms and (4) typically you can only run a limited number of DNA fingerprints on a given gel restricting the number of reliable comparisons.

One of the most powerful, rapid, inexpensive, reproducible, and thus popular, approaches for prokaryote classification is DNA sequencing of the 5S or 16S ribosomal DNA (rDNA). Both 5S and 16S ribosomal DNA have highly conserved and highly variable regions interspersed throughout the full DNA sequence. The highly conserved sequences are used as primers to amplify the highly variable regions. Nucleotide

sequences of the highly variable regions can be unique to the strain level, but are often only able to distinguish to the genus level. Nucleotide sequences can be used to determine similarities between organisms. The rDNA sequence of the unknown organism is matched through an algorithm such as BLAST (Thompson et al. 1994) to known DNA sequences in a database, such as the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) or Bio Informatic Bacterial Identification, version 2 (pbil.univ-lyon1.fr/bibi). The ribosomal DNA sequence database is expanding at a rapid rate, and it is becoming the first step in bacterial identification for many laboratories.

Techniques for Quantifying Fungi in Soils and Plant Tissues

Accurate quantification of fungal pathogens in soil and plant tissue was not possible until the development of real-time quantitative PCR techniques. Higuchi et al. (1993) were the first to document DNA amplification with real-time PCR. Since then, numerous molecular applications have been developed which include mRNA expression studies, DNA copy number measurements in genomic or viral DNAs, and expression analysis of specific splice variants of genes (Ginzinger 2002), quantification of human pathogenic bacteria, protozoans, and fungi (Filion et al. 2003), detection and quantification of bacteria and phytopathogenic fungi in plant tissues (Hristova et al. 2001; Schner et al. 2001), plant extracts (Weller et al. 2000), seeds (Filion et al. 2003), soil (Stults et al. 2001; Schena et al. 2002), soil and potato tubers (Cullen et al. 2001; Lees et al. 2002) and potato peels, tuber washings, and soil (Bell et al. 1999). Real-time quantitative PCR has led to better understanding of pathogen distributions and plant-pathogen interactions, and holds much promise for even greater advances in the future.

Real-time quantitative PCR uses a thermal cycler with a 96 or 384 well format equipped with a fluorescence source and detector. Measurements are made by detecting the increase in fluorescence accompanying PCR amplification. Fluorescence is induced by either a laser (ABI Prism 7700, Applied Biosystems, Foster City, CA) or blue-light emitting diode (Lightcycler, Roche Molecular Biochemicals, Mannheim, Germany). When DNA-binding dyes, molecular beacons (Stratagene, La Jolla, CA), hybridization probes, or hydrolysis probes adhere to the target DNA, fluorescence is detected after every PCR cycle.

The different types of fluorescent strategies have varying degrees of selectivity for detecting a target DNA sequence. Fluorescent DNA-binding dyes, such as SYBR Green, are the least selective of the four methods because they intercalate double stranded DNA and can bind to primer-dimers and non-target as well as target DNA. Also, more than one fluorescent molecule can bind to amplified DNA and the amount of fluorescent signal is determined by the mass of double stranded DNA.

Molecular beacons, hybridization, and hydrolysis probes have DNA sequences that complement and bind the target DNA sequence and thus are more selective than fluorescent dyes that can bind to all double-stranded PCR products. Molecular beacons are probes that hybridize to the DNA amplicon. Initially, the molecular beacon takes the shape of a stem-loop structure with a fluorescent marker and quencher at opposite arms. There is no fluorescence in the stem-loop structure because the quencher is in close proximity to the fluorescent molecule allowing the quencher to absorb the fluorescence and release the energy as heat. The nucleic acid sequence in the loop of the molecular beacon is complementary to the DNA amplicon. When the molecular beacon binds to the

DNA amplicon the fluorescent marker and quencher are separated. The distance is adequate to remove fluorescence quenching, and the increase in fluorescence is detected and recorded by the fluorescence detector.

Hybridization probes involve two separate probes. One probe is labeled with the fluorescent dye fluorescein which emits green light when excited. When a fluorophore is nearby, energy is transferred from the excited fluorescein to the fluorophore, which emits a different (red) wavelength. Detection of a target DNA sequence is accomplished by labeling a second probe with the fluorophore, which does not fluoresce when irradiated with the wavelength used for fluorescein excitation. When the two probes are suspended in the PCR mix, the only molecule to fluoresce is fluorescein (fluoresces green). The fluorophore is too distant to be excited by the fluorescein. When the two probes hybridize “tail to head” on the target DNA, the fluorophore is close to the fluorescein, accepts energy from the excited fluorescein, and emits energy as red light.

Hydrolysis probes, such as the TaqMan[®] Assay (Applied Biosystems, Foster City, CA), were designed to detect short target DNA sequences (about 25 nucleotides) that are so short that fluorescence quenching would still occur even after hybridization to the amplicon. As was the case with molecular beacons, TaqMan[®] probes have a fluorescent dye at one end of the probe and a quencher molecule at the other. The probe binds to the target DNA and hydrolysis by the 5' nuclease activity of the DNA polymerase releases both the quencher and the fluorescent molecule. The latter is detected, free from the quenching effects of the quencher.

Research Objectives

The first objective of this research was to identify culturable bacterial endophytes from crown tissue of bermudagrass, comparing two bermudagrass cultivars: Midlawn, SDS resistant, and Tifgreen, SDS susceptible. The effects of *O. herpotricha* infection on the culturable endophytes were also investigated. A diverse assortment of bacteria capable of colonizing bermudagrass offers greater potential for developing approaches for biological control of SDS, particularly if some of these endophytes express antifungal properties.

The second research objective was to individually assess *in vitro* antagonism of each bacterial endophyte towards the SDS fungus, *O. herpotricha*, in the hope of finding a candidate(s) that can be developed as a biological control agent. The development of a biological control agent for SDS is needed because traditional methods: resistant cultivars, fungicides, and turf management practices, were not successful in controlling SDS in Oklahoma and Kansas.

The third research objective was to develop a real-time PCR assay with TaqMan[®] chemistry to detect and quantify *O. herpotricha* DNA in plant and soil samples to quantify this pathogen. Such information would be useful in studies of the development and spread of SDS.

The fourth research objective was to use the real-time PCR assay to determine if there is a relationship between *O. herpotricha* infection and the resistance of bermudagrass cultivars and to determine the spatial distribution of *O. herpotricha* in plant and soil samples. The data gleaned from the real-time PCR assay with TaqMan[®]

chemistry can lead to a better understanding about the relationship between SDS and bermudagrass.

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CULTURABLE BACTERIAL ENDOPHYTES FROM SPRING DEAD SPOT RESISTANT AND SUSCEPTIBLE BERMUDAGRASS CULTIVARS AND THEIR ANTIFUNGAL PROPERTIES

ABSTRACT

Spring dead spot (SDS) is a devastating fungal disease of bermudagrass. The causal agents are *Ophiosphaerella korrae*, *O. herpotricha*, and *O. narmari*. These *Ophiosphaerella* spp. are soilborne, root-infecting fungi that live off of plant derived nutrients. Traditional pathogen control methods including the use of resistant bermudagrass cultivars, fungicides, and specific cultivation practices, have found limited success in controlling SDS in Oklahoma and Kansas. Biological control agents for SDS have yet to be developed. In hopes of finding culturable bacterial endophytes for development into biological control agents against SDS, bacterial endophytes were isolated from the crown tissue and rhizomes of SDS resistant Midlawn and susceptible Tifgreen bermudagrass cultivars, including SDS infected and non-infected plants. The Log₁₀CFU/g fresh wt was similar for non-infected Midlawn, infected Midlawn, and non-infected Tifgreen plants. Infection with *O. herpotricha* was lower in the Log₁₀CFU/g fresh wt. in infected Tifgreen plants when compared to non-infected Tifgreen plants. Endophytic bacteria were putatively identified to genera by sequencing contigs of their 16S rDNA and BLAST matching these sequences to the NCBI database. Seventy-seven Gram-negative and 51 Gram-positive culturable bacterial endophytes were sequenced. *Microbacterium* was the most frequently isolated genus from all 4 treatments followed by *Acidovorax*, *Stenotrophomonas*, and *Curtobacterium* isolated from 3 treatments. This is

the first report, to the best of my knowledge, of a *Geodermatophilus* sp. and an *Amycolatopsis* sp. as plant endophytes. Thirty-one culturable bacterial endophytes displayed *in vitro* antifungal activity toward *O. herpotricha*. There were more *Pseudomonas* and *Stenotrophomonas* antagonistic isolates than other antifungal genera. This is the first of *Chryseobacterium* sp. with *in vitro* antifungal attributes to the best of my knowledge.

INTRODUCTION

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is a widely distributed warm-season, perennial, sod-forming turf and forage grass (Johns 2004; Turgeon 2005). It can tolerate a wide range of soil types and climatic conditions and forms coarse- to fine-textured turf and loose or dense sods (Fry and Huang 2004, Johns 2004). These attributes have popularized bermudagrass in the sunbelt of the United States and in Australia, New Zealand, and in tropical and subtropical regions of the world. The cultivars Midlawn and Tifgreen are hybrids of *C. dactylon* (L.) Pers. and the South African bermudagrass, *C. transvaalensis* Burt-Davy. In contrast to *C. dactylon*, *C. transvaalensis* is found in a narrow geographic location being confined to the Transvaal and Orange areas of South Africa. *C. transvaalensis* plants are smaller, and produce a fine textured, higher density sod than the typical *Cynodon* sp. (Taliaferro 1992).

Three closely related *Ophiosphaerella* Spegazzini 1909 spp., *O. korrae* (J. C. Walker & A. M. Smith) Shoemaker & C. E. Babcock, *O. herpotricha* (Fr.:Fr.) J. C. Walker, and *O. narmari* Wetzel, Hulbert & Tisserat, the causal agents of Spring Dead Spot (SDS), are the greatest fungal threats to bermudagrass (Sauer et al. 1993; Taliaferro

1995). In the United States, SDS is a widespread disease confined to the northern limits of bermudagrass adaptation where autumn and winter temperatures induce dormancy in these plants (Turgeon 2005).

Ophiosphaerella spp. are soilborne, ectotrophic root-infecting fungi that live off of plant derived nutrients. Cool soil temperatures between 15 to 25 °C are optimal for SDS development (Fermanian et al. 2003). *O. herpotricha* is the main agent of SDS in Oklahoma (Tisserat et al. 2003). Though its primary host is bermudagrass, *O. herpotricha* also causes a patch disease in zoysiagrass (*Zoysia japonica* Steud.) and SDS in buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) (Green II et al. 1993; Dernoeden 1999).

Several methods are used with limited success to control SDS in bermudagrass. Fungicides effective in Alabama, Kentucky, Maryland, and Texas are ineffective in controlling SDS in Kansas and Oklahoma (Anonymous 1999; Vincelli and Powell 2000; Dernoeden 2000; Hagan 1997; Martin and Hudgins 1998). Common alternatives to fungicides include selective cultivation practices and the use of SDS resistant bermudagrass cultivars. Turf management practices that help limit SDS include annual removal of excess thatch and core aerification, fertilization with ammonium sulfate and ammonium chloride with phosphorus and potassium during the growing season and ceasing 6 weeks prior to dormancy of bermudagrass (Watschke et al. 1995, Duble 1996, Fry and Huang 2004). Curiously, more intensively managed turfs are more susceptible to SDS (Shurtleff et al. 1987; Emmons 1995). Heavy applications of organic and inorganic fertilizers, nitrogen, phosphate, and potassium through the growing season and fertilizing after the first week in September will promote the growth of spring dead spot pathogens

(Emmonds 1995, Madison 1971). The use of resistant varieties is another alternative method for controlling SDS. Unfortunately, there are no completely resistant varieties of bermudagrass, although several promising lines are being currently developed. The most resistant cultivars include: ‘Patriot’ (OKC 18-4) [*Cynodon dactylon* L. (Pers.) X *C. transvaalensis* Burt-Davies], Midlawn, and Yukon, but resistance is only partial and may be overcome in severe SDS out-breaks.

One of the most SDS resistant bermudagrass cultivars is the vegetatively propagated cultivar, Midlawn, which was released in 1991 by the Kansas and Oklahoma Agricultural Experiment Stations (Alderson and Sharp 1994). Among seeded varieties cultivars Yukon and Riviera, the most resistant to SDS, were developed and released by Dr. Charles Taliaferro of the Oklahoma State Agricultural Experiment Station (Taliaferro et al. 2003; Taliaferro, personal communications). Susceptible cultivars include some high quality vegetatively propagated types, such as: Tifgreen and Tifway. Tifgreen is an F₁ hybrid between *C. dactylon* collected from the fourth green at the Charlotte Country Club in North Carolina and *C. transvaalensis* from East Lakes Golf course in Atlanta, GA. Tifgreen was released in 1956 by the Georgia Agricultural Experiment Station (Burton 1991). Tifway, a natural hybrid (*C. dactylon* X *C. transvaalensis*) was fortuitously found in 1954 from a seed lot of *C. transvaalensis* from Johannesburg, South Africa and released in 1960 by the Georgia Coastal Plain Experimental Station and Plant Science Research Division, ARS (Burton 1991; Alderson and Sharp 1994).

Biological control is a widely accepted approach for controlling agricultural pests. This option is thought to be more environmentally friendly when compared to chemical fungicide treatments because biological control depends on natural processes. Another

advantage of biocontrol is that it may be less labor intensive and expensive when compared to alterations in cultivation practices. Furthermore, the development of biological control agents can require less time and expense compared to organic fungicides (Handelsman 2002). To further improve the effectiveness of the disease control, biological control agents may be used in conjunction with other disease control practices in an integrated pest management scheme (Vasudevan et al. 2002). Several bacterial biocontrol agents, isolated from soil and diseased tissue, are now currently used to control soilborne crop diseases (Sivan and Chet 1992; Deacon and Berry 1993; Jeger 2001).

Microorganisms that spend part or all of their life cycle inside a plant host without causing disease symptoms are called endophytes. Endophytes that possess antifungal activity may serve as valuable resources as potential biological control agents. Bacterial endophytes are found in almost all plants species, including monocots, dicots, and conifers (Knutson 1973; Patriquin and Döbereiner 1978; Shishido et al. 1995; McInroy and Kloepper 1995a) as well as brackish water and marine plants, ferns, and thylloid bryophytes (McClung et al. 1983a; Kaplan and Peters 1998; Costa et al. 2001; Lovell 2002). Bacterial endophytes are found throughout the plant in an assortment of plant organs, such as leaves, stems, crowns, and roots. At the tissue level they are known to inhabit plant cortical and vascular tissues (Philipson and Blair 1957; Mundt and Hinkle 1976; Gagné et al. 1987; Dunleavy 1989).

Endophytes may function to enhance the development and well being of the plant. Examples of bacterial endophytes include nitrogen fixing bacteria (Sundaram et al. 1988; Suman et al. 2001; Hurek et al. 2002), bacteria with proven antagonistic attributes toward

fungus pathogens (Sturz et al. 1999; Adhikari et al. 2001; Coombs et al. 2003), bacteria that promote growth and development of plants and bacteria that induce systemic resistance in plants (Benhamou et al. 1996).

Studies of bacterial endophytes of graminaceous plants have concentrated on economically important grasses, such as maize, rice, sugar cane, and sorghum (Patriquin and Döbereiner 1978; Dong et al. 1994; Mukhopadhyay et al. 1996; Zinniel et al. 2002). Investigations of bacterial endophytes of less economically important grasses have been fewer in number. Examples include: endophytic bacteria in the sea grass *Halodule wrightii* Ascherson (Kusel et al. 1999) and endophytes from seeds and roots of turfgrasses (Sundaram et al. 1988). The associations of endophytes with various cell types in roots of grasses from Brazil was documented by Patriquin and Döbereiner (1978) using light microscopy. Diazotrophic endophytic bacteria “fix” dinitrogen and benefit plant and endophytes alike. Nitrogen fixing endophytic bacteria have been isolated from turfgrasses (Sundaram et al. 1988), Kallar grass (*Leptochloa fusca* (Linn.) Kunth) (Reinhold-Hurek et al. 1986; Hurek et al. 2002), and a Chesapeake Bay salt marsh grass, *Spartina alterniflora* Loisel (McClung et al. 1983b). However, studies of biological control in turfgrasses have mainly involved manipulation of rhizosphere bacteria and not endophytic bacteria (Nelson and Craft 1992; Zhang and Yuen 2000).

Bacterial endophytes that are antagonistic toward plant pathogens may constitute some of the most promising and versatile biological control agents because they survive in the surrounding plant rhizosphere, readily enter and colonize the host (Kageyama et al. 1992; Pleban et al. 1995; Tsuda et al. 2001), and retain their effectiveness over a period of time (Fravel 2000; Ritter 2003).

This thesis is the first study to document and putatively identify the culturable bacterial endophytes isolated from surface sterilized crown tissue of two cultivars of bermudagrass and to test these endophytes for antagonistic properties against the spring dead spot soilborne fungal pathogen, *O. herpotricha*. A major goal of this effort has been to identify promising candidates for use as a biological control agent.

MATERIALS AND METHODS

Plant Materials

The turfgrass plots were established in September 1997 and managed by the Oklahoma State University Turfgrass Research Center, Stillwater, Oklahoma, under the direction of Dr. Dennis Martin. SDS resistant Midlawn and susceptible Tifgreen cultivars of bermudagrass were used in this study. The turf plots were inoculated on September 25, 1997 using 5 *O. herpotricha* OK188-infected oat grains per inoculation site.

One sampling for characterization of endophytic bacteria was performed in the fall from these plots when it was thought that the disease was most active. Location of a diseased area was determined the previous spring based on the location of a patch of dead turf. The center of the patch was marked with a metal coin buried several inches within the sod at the time of inoculation. The marker-coin was necessary because during the summer months the neighboring bermudagrass recolonized the patch making it disappear. During autumn sampling, the coin marker was found using a standard metal detector. Turf plugs were removed by inserting a metal 2.5 cm diameter X ten cm tall turf plug remover five cm into the turf. When the turf plug remover was removed from the turf,

the turf-soil core, 2.5 cm diameter X ten cm tall, was removed to a separate zip-lock plastic bag.

There were four sampling groups, 1) non-infected Midlawn, 2) non-infected Tifgreen, 3) SDS infected Midlawn, and 4) SDS infected Tifgreen. The plugs from non-infected Midlawn and Tifgreen were harvested from the non-inoculated portion of the turf plot well away from the previously inoculated locations. The plugs from SDS infected Midlawn and Tifgreen were harvested from the edge of what was the spring visible patch, on November 11, 2001.

Three 2.5 cm diameter X five cm deep cores were removed from each of the four treatment plots, packaged in plastic bags, placed on ice, and transported to the laboratory. Within an hour of collection, all plugs were gently washed with sterile Nanopure water to remove soil and dead sheaths from the crown tissue and rhizomes. During processing, the shoots and roots were removed, and the crown tissue and rhizomes were pooled and mixed into one sample for each treatment. When processing was completed, each of the four pooled and mixed treatments, Midlawn non-infected, Midlawn infected, Tifgreen non-infected, and Tifgreen infected, were divided into three replicates each for a total of 12 samples (Table 14). Each replicate was rinsed again in Nanopure water, blotted dry with a paper towel, weighed, and placed into individual 125 mL Erlenmeyer flasks for surface sterilization.

Surface Sterilization

All procedures were performed in a laminar flow hood using aseptic techniques. Nanopure water was filter-sterilized, then autoclaved, to eliminate extraneous bacteria. Glassware, growth media, glass beads, toothpicks, and all other implements were

sterilized prior to use. Procedures from collecting the bermudagrass plugs to plating the sterilized plant homogenate were conducted during the same day for all four treatments.

The tissue samples were pre-washed once in 125 mL Erlenmeyer flasks with 25 mL of sterile phosphate buffered saline (PBS) (McClung et al. 1983a; Barraquio et al. 1997). The PBS was decanted from the flasks and fresh PBS with 0.05% Tween 20 and 5.0 g of 2.5 mm glass beads (Barraquio et al. 1997) were added to the flask. The flasks were placed on a shaker table at 150 rpm for 30 minutes at room temperature. The liquid was decanted and the plant material was washed twice with sterile PBS to remove the detergent. The plant material was separated from the glass beads and transferred to another 125 mL flask with 50 mL of 70 % EtOH (McClung et al. 1983a). The flasks were placed on a shaker table at 100 rpm for 20 min. The 70 % EtOH solution was then removed and the plant material was rinsed twice with Nanopure water. Fifty mL of full strength commercial 6 % sodium hypochlorite bleach (Sturz et al. 1998) containing 0.05 % Tween 20 was added to the plant material in the flask. The flasks were placed on a shaker table at 100 rpm for 20 min. The bleach-Tween 20 solution was removed and the plant material was rinsed five times with Nanopure water, and immediately checked for culturable surface bacteria as described below.

Sterility Control Plates

Three rhizome/crown tissue samples per replicate were removed from the Erlenmeyer flasks, blotted and rolled onto the surface of tryptic soy agar (TSA) (Fluka 22092 tryptic soy broth or Becton Dickinson trypticase soy broth, St. Louis, MO) plates, one plate per replicate. Plant materials were returned to the same Erlenmeyer flasks. The sterility control plates were sealed with two layers of Parafilm[®], wrapped in aluminum

foil to replicate the below ground environment, and incubated upside down at room temperature. Plates were inspected daily for bacterial growth for 10 days. Only two of 12 sterility control plates yielded bacterial growth: Midlawn non-infected replicate three and Tifgreen infected replicate two sterility control plates produced one bacterial colony each indicating surface sterilization was extensive but not complete. As a result, all plates streaked with plant homogenates for Tifgreen infected replicate two and Midlawn non-infected replicate three were discarded.

Isolation of Culturable Endophytic Bacteria

All inoculated agar plates were sealed with two layers of Parafilm[®], wrapped in aluminum foil, and incubated upside down at room temperature. The plates were checked daily for bacterial growth. The surface sterilized plant material was homogenized separately for each replicate in a Waring blender with 70 mL of PBS for 1 min. Serial dilutions of 0.5, 10^{-1} , and 10^{-2} were made and 100 μ L of each were spread with a glass rod dipped in 70 % EtOH, flamed, and cooled, on three 1 X media: TSA (Gardner et al. 1982; Shishido et al. 1995), potato dextrose agar (PDA) (Sigma, St. Louis, MO) and nutrient agar (NA) (Becton Dickinson, Cockeysville, MD) plates. These agar plates are referred to as spread plates. Bacterial growth was noted 3 to 7 days after inoculation on most spread plates, although some did not develop any colonies even after 10 days. All visible colonies were picked from the spread plates. A total of 1466 visible colonies were removed using sterile toothpicks, removing either the entire colony or portions of each colony (Table 15). Each picked colony was then placed into individual 1.7 mL microcentrifuge tubes containing one mL of liquid broth corresponding to the single medium used for the spread plates. These tubes were placed into cardboard boxes,

approximately 15 cm square X four cm tall, with microcentrifuge tube dividers. The lids were placed onto the box to mimic the underground environment, and the box was incubated at room temperature until visible growth of bacteria was evident, approximately two days.

To produce pure cultures, a subset totaling 130 colonies was randomly picked from 4 treatments (Table 15). The initial bacterial liquid cultures, see above paragraph, were serially streaked three times for isolation onto the same agar medium as the liquid medium. Some serially streaked plates developed colonies with two or more different morphologies. These colonies were picked and assigned an alpha-numeric identification number to associate it with its parent colony and were streaked three times in successive agar plates to produce pure cultures. A total of 89 colonies were produced from streaking the 130 original colonies for pure cultures. These 89 colonies were added to the 130 original colonies bringing the total to 219 pure cultures. These 219 cultures were individually used to inoculate individual 1.7 mL microcentrifuge tubes with one mL of nutrient broth, incubated for approximately two days, and stored for later use (Table 15).

Extraction of DNA from Bacterial Cultures

For each of the 219 endophytes replicate extractions were performed. Five mL of each pure endophyte culture were inoculated into 50 mL tubes containing five mL of tryptic soy broth. The test tube was incubated at room temperature on a shaker table at 65 rpm. After one to five days, when bacterial growth was evident, two aliquots of 1.5 mL were removed from each test tube, placed into separate 1.7 mL microcentrifuge tubes, and centrifuged at 6000 g for one min. After discarding the supernatant, the pellet was resuspended in 1.5 mL of PBS, centrifuged at 6000 g for one min and then the

supernatant was discarded. This wash step was repeated three times. After discarding the supernatants, the pellet was washed in one mL of 1 M NaCl and centrifuged at 6000 g for one min. The supernatant was discarded and the pellet was resuspended in 100 μ L of Nanopure water. The two resuspended replicate pellets for each isolate were combined into one 1.7 mL microcentrifuge tube and stored on ice for the next step. The combined pellet suspension of 70 μ L was removed to a 2 mL boiling tube, a screw-capped tube with rubber O-ring in cap (United Scientific Products, San Leandro, CA), with 1.0 mg of 0.10 mm glass beads (Biospec Products, Inc., Bartlesville, OK). The boiling tube was shaken for 1 min at 1400 rpm in the bead beater (Biospec Products, Inc., Bartlesville, OK) then placed on ice. The boiling tube was centrifuged at 6000 g for one min and the supernatant containing the isolated DNA was removed to a fresh 1.7 mL microcentrifuge tube and stored at -20°C .

PCR Amplification, PCR Product Cleanup, and 16S rDNA Sequencing

The 16S rDNA of the 219 culturable bacteria was amplified using the Qiagen Taq DNA Polymerase according to manufacturer's instructions (Qiagen, Valencia, CA). PCR primers were synthesized by Integrated DNA Technologies (Coralville, ID). The PCR master mix consisted of: Qiagen Buffer (1X), MgCl_2 (3 mM), dNTP (0.2 mM each), forward primer 5'-CAG CAG CCG CGG TAA TA-3' (250 nM), reverse primer 5'-CAA CAT CTC ACG ACA CGA GC-3' (250 nM), and Taq DNA polymerase (1U/100 μ L). The PCR reaction was run in a MJ PTC-200 (MJ Research, Watertown MA) thermal cycler with a program as follows: initial denaturation at 94°C for three min, cycle denaturation at 94°C for one min, cycle annealing at 52°C for 30 sec, cycle extension at 72°C for one min, repeat cycle steps 34 more times, final extension 72°C for ten min,

and then hold at 4 ° C. The tubes were placed in the thermal cycler at 94 ° C to “Hot Start” the reaction.

The PCR amplifications were checked by gel electrophoresis using a 2 % agarose gel, 0.5 X tris-borate EDTA buffer for running buffer, and six µL of PCR product (Sambrook and Russell 2001). The electrophoresis was conducted at 8 V/cm, at room temperature, until the tracking dye migrated approximately 3/4 the length of the gel. The DNA bands were photographed under long wave ultraviolet light using a Bio-Rad Gel Doc system (Bio-Rad Laboratories, Hercules, CA).

The unused primer and dNTPs were removed from the PCR products using a 96 well MultiScreen HV plate (Millipore Corp., Billerica, MA) loaded with hydrated Sephadex G-50 beads (Amersham Pharmacia, Piscataway, NY). A maximum of 20 µL of PCR product was added to the center of each well and centrifuged at 3000 g at room temperature for 5 min into a collection plate. The filtrates in the collection plate contained the purified PCR product for sequencing. The collection plate was covered with plastic adhesive backed tape and stored at –20 °C until sequencing. The DNA sequencing was performed at the Recombinant DNA/Protein Core Facility, Oklahoma State University, Stillwater, OK.

DNA Sequence Analysis

The 219 bacterial endophyte DNA sequences were trimmed of uncertain bases and vector sequences (N) at the 5' and 3' ends generating sequences between 483 and 963 bases long. Fifty endophyte sequences of low quality (greater than 1.0 % N, fewer than 500 bases) were discarded, leaving 169 high quality DNA sequences from the 219 bacteria (Table 15).

Endophyte Putative Identification

Endophyte DNA sequences were compared to known 16S ribosomal sequences using BLAST (Altschul et al. 1997) algorithms from the nonredundant nucleotide sequences in the National Center for Biotechnology Information (NCBI) database. The search consisted of pairwise comparisons using the low complexity filter for bacteria only and the 10 best matches were selected. Putative identifications of endophyte taxa were made using several scenarios (Table 16).

Alignment of the 16S rDNA Contig Sequence using ClustalX 1.8

All 16S rDNA sequences were aligned using ClustalX 1.8 software for Macintosh computers (Thompson et al. 1997). The parameters for the pairwise alignment were set by selecting 10.0 for gap opening, 0.1 for gap extension, and IUB for the DNA Weight Matrix. Hall's (2001) suggestion to set the pairwise alignment and multiple alignment parameters to the same settings even though only one of the two alignments were to be selected was followed. The parameters for the multiple alignment were 10.0 for gap opening, 0.1 for gap extension, 30 for delay divergent sequences (%), 0.50 for DNA transition weight [0-1], use negative matrix, and select IUB for DNA weight matrix.

De-Replication of Endophytic Bacteria Putatively Identified as *Microbacterium*

By far the most abundant genus found was *Microbacterium* (Orla-Jensen 1919) Takeuchi & Hatano 1998, comprising 71 isolates out of the 169 sequences representing 42% of all isolates (Table 15). Seventy-one putatively identified *Microbacterium* 16S rDNA contig sequences were aligned using ClustalX 1.8 to determine if the clones had identical sequences (Table 15). The alignment included the 71 *Microbacterium*, the *Escherichia coli* (Migula 1895) Castellani & Chalmers 1919, and the *E. coli* 0157:H7

16S rDNA gene sequence to act as a guide in truncating the aligned sequences to homologous regions in the 16S gene. The aligned sequences were truncated at the 5' end at nucleotide number 534, and at the 3' end at nucleotide number 1019 of the *E. coli* 0157:H7 gene sequence. The alignment produced 5 sets of putative clonally derived 16S rDNA contig sequences and one set of 25 distinguishable 16S rDNA contig sequences. One contig sequence from each of the 5 sets of indistinguishable sequences was randomly selected and added to the 25 distinguishable sequences to yield 30 unique *Microbacterium* 16S rDNA contig sequences (Table 15). This reduction of 41 *Microbacterium* sequences resulted in a total of 128 unique culturable bacterial endophyte sequences for analysis.

Cladogram of 128 Endophytic Bacterial Isolates, *Bacillus megaterium*, and *E. coli* 0157:H7

An alignment of 16S rDNA contig sequences from 128 endophytic bacterial isolates was performed (Table 15). Database 16S rDNA sequences from *B. megaterium* de Bary 1884 (AY030338 (Venkateswaran et al. 2003)) and *E. coli* 0157:H7 (NCBI accession number AY513502 (Gee et al. 2004)) were included in the alignment as representatives of Gram-positive and Gram-negative bacteria. The aligned sequences were truncated at the 5' end corresponding to *E. coli* 0157:H7 16S rDNA nucleotide number 465 and at the 3' end corresponding to nucleotide number 1041.

All cladograms were neighbor-joining bootstrapped (1000 X). Phylip software version 3.573c (Felsenstein 1989) was used to generate the neighbor-joining bootstrapped tree. TreeView PPC 1.6.6 software (Development) (Page 1996) was used to view the tree. For all cladograms, branches with bootstrap values less than or equal to 500 were

collapsed. A neighbor-joining bootstrapped (1000 X) cladogram was constructed, as mentioned earlier, from the truncated alignment and the internal branches were labeled to class using the putative identifications of the endophytic bacteria. The cladogram was divided into clades by dissecting the classes. One endophyte representative was randomly chosen from each homogeneous clade of the neighbor-joining bootstrapped (1000 X) cladogram of the 128 isolates. In the case of heterogeneous clades one member of each taxon was randomly chosen as collective representatives of that clade.

The Selection of Type Species from the NCBI Database

The web-based List of Bacterial Names with Standing in Nomenclature (<http://www.bacterio.cict.fr>) was searched for the type species of each putatively identified genus and for the strain identification numbers, e. g. American Type Culture Collection (ATCC) (www.atcc.org). The NCBI database was searched to find the 16S rDNA sequences of the type species. All but two clade representative endophytic bacteria, *Afipia* Brenner et al. 1992 emend. La Scola et al. 2002 and *Mycobacterium* Lehmann & Neumann 1896, had their type species 16S rDNA gene in the NCBI database. The type species for *Afipia* is *A. felis* Brenner et al. 1992 (ATCC53690). There are several *A. felis* strains with 16S rDNA genes partially sequenced and the longest available sequence (NCBI accession number AF338177 (van Berkum and Eardly 2002), ATCC49715) was chosen. The type species of *Mycobacterium* is *M. tuberculosis* (Zopf 1883) Lehmann & Neumann 1896 (ATCC27294). *M. tuberculosis* strain H37/Rv (NCBI accession number X55588 (Wolters 1990) is a member of the type species. Its genome is completely sequenced and the 16S rDNA sequence from this strain was used.

Alignment of the 16S rDNA Contig Sequences

The 16S rDNA sequences for 25 endophyte representatives, 17 type species references, *Afipia* (A338177), *Mycobacterium* (X55588), and *B. megaterium* were aligned using Clustal X 1.8. The alignment was truncated to yield a fragment from nucleotides 570 to 1218 according to the *B. megaterium* 16S rDNA sequence. A neighbor-joining bootstrapped (1000 X) cladogram was generated from the truncated alignment of the 45 16S rDNA contig sequences using the aforementioned parameters.

O. herpotricha Antagonism Assay

O. herpotricha KS strain 188 was acquired from Ned Tisserat of Colorado State University and maintained on autoclaved oats (Ag Center, Stillwater, OK). The autoclaved oats were prepared in one liter glass jars with screw-lids filled half way with whole oats and 250 mL of Nanopure water. The jars were autoclaved then cooled. One jar of autoclaved oats was inoculated with one 1 cm diameter plug of *O. herpotricha* hyphae and agar removed from the leading edge of actively growing hyphae on PDA.

The assay to measure the antagonism of culturable endophytic bacteria to *O. herpotricha* was performed in duplicate on 6 different media, 1/5X and 1X NA, PDA, and TSA, to determine if *in vitro* antagonism is nutrient dependent. A 1 cm diameter plug of *O. herpotricha* hyphae and agar was removed from the actively growing leading edge of the *O. herpotricha* fungus on PDA. The plug was placed onto the center of 150 mm diameter medium plates, one per plate. The plates were ready for bacterial inoculation when the hyphae grew half the distance to the edge of the plate. Each plate was marked to delineate 16 equal wedges and two μ L of each of 16 pure endophyte cultures grown in tryptic soy broth were inoculated onto the surface of the agar two mm

from the leading edge of the fungus. The drops were allowed to dry in the covered plate inside the laminar flow hood. Antagonism was assessed after the fungus grew beyond and between the bacterial colonies.

Antagonism was measured by assigning a numerical score. A score of zero indicated no antagonism; the fungus grew all the way through the bacterial culture with visible hyphae extending beyond the bacterial culture in the agar. A score of one indicated a slight retardation in the leading edge of hyphae: the fungus grew through the bacterial colony, but did not extend beyond the bacterial colony. Score of two indicated the leading edge of the fungus grew two thirds of the distance through the bacterial colonies. Three indicated the leading edge grew one third of the distance through the bacterial culture. Four indicated the leading edge touched the edge of the bacterial colony but did not grow into the colony. Five indicated a zone with no fungal or bacterial growth between the leading edge of the fungus and the edge of the bacterial colony.

RESULTS

The first phase of this study compared bacterial endophyte diversity in two bermudagrass cultivars, SDS resistant Midlawn and susceptible Tifgreen. The four treatments were *O. herpotricha* infected and non-infected Midlawn and Tifgreen plants. A total of 1466 isolates encompassing all treatments (Table 14) were recovered from our samples. Non-infected Midlawn plants generated the greatest number of culturable bacterial endophytes whereas the least number was isolated from infected Tifgreen followed closely by infected Midlawn (Table 15). One replicate from Midlawn non-

infected plants stood out among all agar plates, as it yielded the greatest number of endophyte colonies (Table 14). The geometric mean of replicate measurements of culturable bacterial counts (\log_{10} CFU g⁻¹) per treatment were Midlawn non-infected, 5.2; Midlawn infected, 5.1; Tifgreen non-infected, 5.0; and Tifgreen infected, 4.5.

To study the endophyte taxa richness in the four treatments, we originally aimed to select 50 isolates per treatment. However, two treatments, infected Midlawn and Tifgreen, gave fewer than 50 colonies from all plates combined. In addition, owing to an oversight, fewer colonies were selected from some treatments than planned (Table 15). When the selected colonies were triple-streak purified additional bacterial isolates were present. The final total of 128 culturable bacterial endophytes were distributed as follows: Midlawn non-infected with 32 isolates, Midlawn infected with 19 isolates, Tifgreen non-infected with 50 isolates, and Tifgreen infected with 27 isolates (Table 15).

The 128 culturable bacterial endophytes were putatively classified by matching their 16S rDNA c sequence against the NCBI database using the BLAST algorithm. In addition, the 128 bacterial endophytes were assigned to major categories, groups, phyla, classes, and genera as described in Bergey's Manual of Systematic Bacteriology 9th edition (Holt et al. 1994) using the guidelines listed in Table 16. Of the 128 endophytes, 77 belonged to Major Category I, the gram-negative rods and cocci with cell walls, and 51 endophytes belonged to Major Category II, the gram-positive rods and cocci with cell walls (Table 17). The Major Category I contained 11 putatively identified genera. The most frequently isolated Major Category I genus was *Acidovorax*, followed by *Stenotrophomonas* and *Pseudomonas*. The genera with the fewest members were *Sphingomonas*, *Pantoea*, and *Rhizobium* (Table 17). The Major Category II contained 7

genera with *Microbacterium* and *Curtobacterium* the most frequently isolated genera. The genera with the fewest numbers were *Amycolatopsis*, *Geodermatophilus*, *Mycobacterium*, and *Staphylococcus*.

Assessing the diversity of endophyte taxa of the four treatments was an integral part of this experiment because the difference in genera richness may be cultivar and disease dependent. Eighteen genera and six broad taxa were isolated from the four treatments. Broad taxa of class, family, or group were assigned to those isolates whose genus classification was uncertain. There was a positive relationship between the number of CFUs in each treatment and the number of different taxa in each treatment ($R^2=0.78$). Non-infected plants displayed a greater diversity of genera and CFUs than diseased plants. Fourteen genera and five broad taxa were isolated from non-infected Midlawn and Tifgreen plants and 11 genera and three broad taxa were isolated from the infected plants (Table 18). Susceptible Tifgreen displayed a greater diversity of genera and CFUs than resistant Midlawn (Table 18). Sixteen genera and six broad taxa were isolated from non-infected and infected Tifgreen plants and nine genera and three broad taxa were isolated from non-infected and infected Midlawn plants. Only one genus, *Microbacterium*, was isolated from all 4 treatments.

The similarities of the 128 16S rDNA endophyte sequences were assessed by using ClustalX 1.8 software to align these sequences including the 16S rDNA reference sequences from *B. megaterium* and *E. coli*. A neighbor-joining bootstrapped (1000 X) cladogram was constructed and the algorithm grouped the endophyte sequences into the classes *Actinobacteria*, “*Bacilli*”, “*Flavobacteria*”, “*Alphaproteobacteria*”, “*Betaproteobacteria*”, and “*Gammaproteobacteria*” (Figs 1-5). The class *Actinobacteria*

was divided into the most clades and contained the largest number bacterial endophyte isolates (Figs. 1, 4 and 5). The class with the fewest isolates and thus smallest clade was the “*Flavobacteria*” clade (Figs. 1, 4). Of the *Proteobacteria* classes, “*Gammaproteobacteria*” was divided into two separate clades and contained the greatest number of bacterial isolates followed by the separate clades of “*Alphaproteobacteria*” and “*Betaproteobacteria*” (Figs. 2, 3).

A second neighbor-joining bootstrapped (1000 X) cladogram was constructed with the 16S rDNA sequences of clade representative endophytes and their type species or closely related strains to demonstrate how representative sequences would group with known taxa (Fig. 6). The neighbor-joining algorithm paired 11 endophyte representatives with their type species (Fig. 6). However, 8 of the putatively identified endophytes did not directly pair with known taxa.

The *in vitro* antagonism of 219 culturable bacterial endophytes towards *O. herpotricha* was assayed to assess their potential as antifungal isolates with promising biological control properties. Experiments were conducted to determine the level of endophyte antagonism against *O. herpotricha* in three different laboratory media, NA, PDA, and TSA at two concentrations 1X and 1/5th to determine if *in vitro* antifungal properties were nutrient dependent. *O. herpotricha* hyphal growth was visually measured and grew equally well on 1 X NA, PDA, and TSA media and had a reduced growth rate on the 1/5th NA, PDA, and TSA media. Thirty-one putatively identified bacterial endophytes were antagonistic *in vitro* towards the causal agent of SDS, *O. herpotricha*. These bacterial endophytes displayed different levels of antagonism on different media. In general, full strength media (1 X) supported greater levels of antifungal properties than

the 1/5th media (Table 19). There was a greater number of antifungal isolates from non-infected Midlawn and Tifgreen plants compared to infected Midlawn and Tifgreen plants. The number of isolates from non-infected Midlawn compared to non-infected Tifgreen plants was similar as was the number of isolates from infected Midlawn compared to infected Tifgreen plants. None of the antagonistic endophytes were isolated from all four treatments, nor were all members of any taxon antifungal (Table 20). The 31 antifungal isolates were grouped by the neighbor joining algorithm into the classes *Actinobacteria*, “*Bacilli*”, “*Betaproteobacteria*”, “*Gammaproteobacteria*”, and “*Flavobacteria*” with the two “*Gammaproteobacteria*” clades containing the highest numbers of isolates (Figs. 2-5).

DISCUSSION

Spring dead spot produces circular patches of dead and dying bermudagrass that are visible in the spring. Over the summer, the patches disappear as the bermudagrass recolonizes the infection zone. When lower autumn temperature arrive, bermudagrass enters dormancy and presumably infection occurs (Fermanian et al. 2003). It was during this transition time in late autumn that samples were harvested for this endophyte study. Non-infected plant material showed no signs of necrosis. In infected material only a small percentage, approximately 10 - 20 %, of the underground structures of infected plants of both cultivars had visible black plaques and necrosis. This infrequent occurrence of visible necrosis may be characteristic of a patchy distribution of infection within the root system of infected bermudagrass. So far very little research, if any, has been conducted on SDS distribution in the infection zone within the field. If SDS is

patchy distributed then many more samples should be taken to quantify the level of infection in terms of the frequency of infected tissues compared to non-infected tissues. If this study was to be repeated in the field we would increase the number of samples. We hypothesize that this would provide an adequate comparison among treatments as to the degree of infection. Much more research is necessary on the environmental conditions and the temporal and spatial distribution of infection of SDS causing organisms in the field before an adequate understanding of the disease can be obtained. We have initiated a study to determine the distribution of *O. herpotricha* in patches of dead and dying turf during the spring (Chapter 2 of this thesis); the results generally support the idea of a patchy distribution.

Culturable Bacterial Endophytes

This study is the first to document the diversity of culturable bacterial endophytes from surface sterilized crown tissues of bermudagrass cultivars. In addition, this study presents an original comparison of the diversity of culturable bacterial endophytes in plants infected with the causal agent of SDS, the fungus *O. herpotricha*, and non-infected plants. We focused on the endophytes associated with the crown tissue because the crown tissue is the perenniating tissue for root and shoot initiation and may serve as the distribution point for some of the endophytes found in roots and shoots. Endophytes from other plant species have been shown to migrate into either root (Marti et al. 1999) or shoot tissues (Patriquin et al. 1978; Gardner et al. 1972; Gagné 1987) from the crown.

Abundance of Culturable Bacterial Colonies

There was a high disparity among the number of visible colonies on agar plates spread with serial dilutions of the plant homogenate from different treatments. Infected Midlawn, non-infected Tifgreen, and infected Tifgreen serially diluted plant homogenates produced a total of 36, 124, and 35 visible colonies, respectively (Table 14). In contrast, Midlawn non-infected serial diluted plant homogenate produced the highest number of visible colonies for a total of 1271, with Midlawn replicate 1 contributing 1249 colonies and replicate 2 with 10 colonies and replicate 3 contributed 12 colonies but was discarded because the corresponding sterility control plates were contaminated. The anomalous high number of visible colonies obtained from Midlawn non-infected replicate 1 is at least 20-fold higher than any other replicate, and could be attributed to heterogeneous distribution of endophytes in the harvested plant tissues. The material extracted from homogenized material from replicate 1 probably contained far greater densities of endophytes than all other plant tissue replicates. The differences in colony counts cannot be attributed to variations in the mass of plant tissue as replicate 2 from the same pooled plant material yielded vastly fewer colonies (9 vs. 1029) than replicate 1 (Table 14). Such wide variations in endophyte abundances from replicate samples are common, with some reports demonstrating variations covering 4-6 orders of magnitude (Zinniel et al. 2002; Bell et al. 1995). The reasons underlying such heterogeneity remain to be more fully explored.

Community Ecology of Culturable Bacterial Endophytes

The putatively identified culturable bacterial endophytes isolated in this study are distributed across Major Category I, the gram-negative rods and cocci with cell walls and

Major Category II, the gram-positive rods and cocci with cell walls as described in Bergey's Manual of Systematic Bacteriology 9th edition (Holt et al. 1994) (Table 17).

The 128 bacterial endophytes whose 16S rDNA sequences displayed a positive relationship between the number of CFUs recovered from each treatment and the number of different taxa documented in each treatment ($R^2=0.78$). In other words, those treatments with the higher numbers of culturable CFUs were richer in diversity of endophyte genera.

For the most part, the 18 genera and 6 broad taxa, e. g. *Enterobacter* Hormaeche & Edwards 1969/*Pantoea* Gavini et al. 1989 emend. Mergaret et al. 1993 and *Microbacteriaceae* Park et al. 1995, fall in the range of plant endophytes isolated and identified from various agricultural crops (McInroy and Kloepper 1995b; Sturz et al. 1997; Garbeva et al. 2001) (Table 19). However, there were exceptions to this rule. Some bacteria known to inhabit soils but not plants were identified as bermudagrass endophytes in this study. To our knowledge, this is the first report of endophytes from genera *Amycolatopsis* Lecheralier et al. 1986 and *Geodermatophilus* Luedemann 1968 from any plant species. Both genera are classified under the order *Actinomycetales* Buchanan, 1917. *Amycolatopsis* has been isolated from soils in China, India, Brazil, and Kuwait (Chung et al. 1999; Wink et al. 2003; Semedo et al. 2001; Al-Musallam et al. 2003). Of special interest, *Amycolatopsis* has been shown to produce several antibiotics including the commercial antibiotics vancomycin and rafamycin (Jin et al. 2002; Padma et al. 2002; Krishna et al. 2003; Wink et al. 2003). The genus *Geodermatophilus* contains one described species, *G. obscurus* (Luedemann, 1968). *Geodermatophilus* has been isolated from diverse environments including soils of the Mojave Desert

(California-Nevada, USA), Asgard Range (Transantarctic mountains), and Gardabani raion (sic) (Central Georgia in Asia) (Garrity et al. 1996; Mevs et al. 2000; Kudukhashvili et al. 2001; Dungan et al. 2003). *G. obscurus evereste* has been touted as the highest living bacterium on earth (Moffat, 2004) but this bacterium subspecies name (*evereste*) has no standing in bacterial nomenclature and is found neither in the NCBI database nor the American Type Culture Collection.

The most abundant endophytes in the crown tissue, in terms of number of colonies recovered, belong to the genus *Microbacterium*. This genus was unevenly distributed among 4 treatments. The reasons underlying the reduced number of *Microbacterium* isolates in diseased Midlawn crown tissue are not known. The unique environment associated with diseased Midlawn tissues may have influenced *Microbacterium* growth. Even so, *Microbacterium* is ubiquitous in plants and has been documented in surface sterilized leaves, stems, and roots of several agronomic crops, grasses, and prairie plants as well as soils (McInroy and Kloepper 1995a; Elbeltagy et al. 2000; Chelius and Triplett 2001; Garbeva et al. 2001; Zinniel et al. 2002; Mostafa and Helling 2003; Macur et al. 2004; Zhang et al. 2004). The unfastidious habit of *Microbacterium* may be attributed to its metabolism, which is primarily respiratory but can be weakly fermentative and chemoorganotrophic (Holt et al. 1994).

Comparison of Resistant and Susceptible Cultivars

Midlawn and Tifgreen are resistant and susceptible hybrids, respectively, of *Cynodon dactylon* (L.) Pers. One aim of this investigation has been to determine whether resistant and susceptible cultivars sustain different endophyte communities. The ratio of identified taxa to the number of isolated endophytes was similar in healthy Midlawn and

Tifgreen crown tissues, 0.31 and 0.34 respectively. This measure of endophyte diversity suggests that the diversity and abundance of bacterial endophytes were similar in these resistant and susceptible cultivars. Our findings of no cultivar dependence of endophyte diversity coincide with those of Sturz et al. (1999), who studied 4 potato (*Solanum tuberosum* L.) cultivars and Adams and Kloepper (2002) who investigated 9 cotton (*Gossypium hirsutum* L.) cultivars. In contrast, cultivar differences were found in the works of Sturz and Christie (1999) who studied 4 red clover (*Trifolium pratense* L.) cultivars, and Germida and Siciliano (2001), who compared ancient land races and recent cultivars of rice (*Oryza*).

Endophyte Diversity in Healthy and Diseased Plants

One goal of this study was to determine the impact of disease on the abundance of bacterial endophytes. SDS was not associated with marked effects on the abundance (5.1 vs. 5.2 log₁₀CFU g⁻¹) of bacterial endophytes in infected Midlawn plants compared to non-infected plants, respectively. However, the endophyte abundance in Tifgreen plants exhibited more substantial differences when comparing healthy with diseased plants. Endophyte abundance was lower in infected Tifgreen plants compared to non-infected plants, 4.5 vs. 5.0 log₁₀CFU g⁻¹, respectively.

In vitro Antagonism of Endophytes Towards *O. herpotricha*

There are numerous studies characterizing culturable bacterial endophytes with respect to activity against phytopathogenic organisms (Tervet and Hollis 1948; Knutson 1973; McClung et al. 1983a; Barraquio et al. 1997; Araújo et al. 2002). To our knowledge, our study is the only one that compares the genera richness, abundance, and

antifungal properties of culturable bacterial endophytes between resistant and susceptible cultivars and in healthy and diseased plants.

In vitro antifungal properties of 128 bacterial endophytes were assayed to determine if Midlawn or Tifgreen differed in taxa and abundance of antagonist endophytes. We also wanted to discover and identify suitable endophytes to develop into a biological control agent for SDS. Ten endophyte taxa displayed significant *in vitro* antagonism towards *O. herpotricha* (Table 20). A comparison of their antagonism with that of known antifungal bacteria reveals a precedent that certain members of these taxa show significant antifungal properties (Table 21). To our knowledge, we are the first to document the *in vitro* antifungal properties of a *Chryseobacterium* sp. The genus *Chryseobacterium* was described in 1994 by Vandamme et al. and includes some members formerly classified in the genus *Flavobacterium* Bergey et al. 1923. *Chryseobacterium* has been isolated from soils (Radianingtyas et al. 2003; Rosado and Govind 2003; Wery et al. 2003) and natural waters (Arvanitidou et al. 2003). A few *Chryseobacterium* spp. are opportunistic pathogens in humans with compromised immune systems (Bloch et al. 1997; Fraser and Jorjensen 1997).

Non-infected Midlawn and Tifgreen plants contained 87 % of the *in vitro* antagonistic CFUs while 13% of the antagonistic CFUs originated from infected plants (Table 16). Some of this difference can be attributed to the distribution of the 128 isolates among the 4 treatments, with 64% coming from non-infected plants and 36% from infected plants (Table 18). Furthermore, 10 of the 46 isolates from infected plants were identified as *Acidovorax*, and none of the *Acidovorax* isolates showed any antagonism toward *O. herpotricha* (Tables 18 and 20). Both antagonistic and non-

antagonistic members were found within 9 of the 10 taxa containing antagonistic bacteria. Exceptions were the two *Xanthomonadaceae*; both of which displayed *in vitro* antagonism towards *O. herpotricha*. Sturz et al. (1998) reported similar findings of antagonistic and non-antagonistic members within endophyte species: 1 of 2 *Bacillus brevis* Migula 1900 isolates and 2 of 7 *Pseudomonas chichorii* (Swingle 1925) Stapp 1928 displayed *in vitro* antagonism towards *Rhizoctonia solani* Kuhn.

The *in vitro* antagonism of the antifungal bacterial endophytes in this study might be attributed to competition for nutrients between *O. herpotricha* and the individual bacterium. This kind of *in vitro* assay does not distinguish effects of nutrient depletion from production of antimicrobial metabolites. Growth medium influenced the level of *in vitro* antagonism with 1 X TSA sustaining the greatest and 20 % NA the least antagonism, as a rule, over all isolates tested (Table 19). This suggests the nutritional environment is important for the expression of antagonism (James and Gutterson 1986; Milner et al. 1996; Duffy and Defago 1999). The nature of the nutritional environment and effect of nutrient composition on endophytic growth in the bermudagrass apoplastic space are yet to be determined. Studies characterizing the bacterial endophyte antagonism towards *O. herpotricha* using natural apoplastic fluids may lead to a greater understanding of the effect of nutrients under conditions that better simulate the natural apoplast environment.

The antifungal culturable bacterial endophytes from this study have potential as biological control agents against *O. herpotricha*. There are precedents for use of bacteria as successful biological control agents for plant diseases; some examples of such commercial products include: Companion (*Bacillus subtilis* (Ehrenberg 1835) Cohn

1872 GB03, Growth Products, White Plains, NY), Subtilex (*B. subtilis*, The MicroBio Group Ltd., Boulder, CO), Spot-Less (*Pseudomonas aureofaciens* Kluyver 1956 TX-1, Eco Soil Systems, Inc., San Diego, CA) (Fravel 2000), and Serenade (*B. subtilis* QST-713, AgraQuest Inc., Davis, CA), the later of which possesses broad range fungicidal activity (Ritter 2003). In our study, we have isolated antifungal members of the genera *Bacillus* Cohn 1872 and *Pseudomonas* Migula 1894, two of the genera most used for biological control purposes. Additional research is ongoing to establish whether one, or a collection, of our antifungal bacterial endophytes could be developed into a biological control agent for *O. herpotricha*.

Bacterial Endophytes as Biological Control Agents

The development of bacterial endophyte(s) into biological control agent(s) for soil fungal phytopathogens poses a challenge. There are distinct advantages of employing the endophyte system for biocontrol purposes, even though few, if any, endophytes have been developed for this purpose. Endophytes are particularly well adapted to thrive within plant tissues, which might contribute to successful biocontrol. The antagonistic endophytic bacteria isolated in this study offer potential as biocontrol agents, but further research is necessary to optimize the colonization and antagonism of these endophytes.

CONCLUSIONS

Seventy-seven Gram-negative and 51 Gram-positive culturable bacterial endophytes, including some with *in vitro* antifungal attributes, were readily isolated from the crown tissue of resistant Midlawn and susceptible Tifgreen cultivars of bermudagrass, infected with *O. herpotricha* and non-infected. This study is the first, to our knowledge,

to document a *Geodermatophilus* sp. and an *Amycolatopsis* sp. as plant endophytes. The diversity of taxa and abundance of bacterial endophytes were similar in healthy and diseased Midlawn crown tissues. In Tifgreen, infected plants exhibited lower endophyte abundance but greater diversity of taxa compared to healthy plants. Antifungal endophytes were abundant in healthy Midlawn and Tifgreen plants, but their abundance was substantially lower in infected plants from both cultivars. We report the first observation, to our knowledge, of *in vitro* antifungal attributes of a *Chryseobacterium* sp.

There are several attributes required for a successful biological control agent for *O. herpotricha*. Ease of culture and long shelf-life are necessary for low-cost commercial production. Ease of inoculation, such as a root dip or application to the soil, makes the product user-friendly. The successful biocontrol agent must have fitness in the rhizosphere, motility to the root, and a method to enter the root. The biocontrol agent must also have fitness inside the root and the ability to display antagonism inside the root.

The abundance and diversity of culturable bacterial endophytes in bermudagrass demonstrate that turfgrasses are good hosts and valuable resources for endophytes with antifungal properties. The cohort of *in vitro* antifungal bacterial endophytes has potential as biological control agents for SDS. The culturable bacterial endophytes in our collection merit further study to elucidate the dynamics of their microbial communities, their classification, taxonomy, and physiological aspects conducive to biological control agents.

Table 14. Replicates, dilutions, and number of culturable bacterial endophyte colonies isolated from surface sterilized crown tissue from 4 experimental treatments of Midlawn and Tifgreen cultivars of bermudagrass. g of tissue = g of crown tissue and rhizomes

Cultivar	Midlawn	Non-infected							
Replication	1			2			3 DID	NOT	USE
g of tissue	0.25 g			0.26 g			0.27 g		
Dilution	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}
Colonies	1029	212	16	9	1	0	3	1	0
Cultivar	Midlawn	Infected							
Replication	1			2			3		
g of tissue	0.15 g			0.15 g			0.14 g		
Dilution	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}
Colonies	20	4	2	6	4	0	44	7	2
Cultivar	Tifgreen	Non-infected							
Replication	1			2			3		
g of tissue	0.37 g			0.40 g			0.44 g		
Dilution	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}
Colonies	59	7	0	8	2	1	50	8	0
Cultivar	Tifgreen	Infected							
Replication	1			2 DID	NOT	USE	3		
g of tissue	0.38 g			0.42 g			0.42 g		
Dilution	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}	0.5 X	10^{-1}	10^{-2}
Colonies	1	0	0	1	0	0	27	3	3

Table 15. The number of culturable bacterial endophytes isolated from the crown tissue of Midlawn and Tifgreen cultivars of bermudagrass. The protocol steps and the number of colonies per treatment at each step.

Protocol Step	Midlawn non-infected	Midlawn infected	Tifgreen non-infected	Tifgreen infected	TOTAL
1. Original colonies picked from spread plates.	1271	36	124	35	1466
2. Selected colonies for study from replicates and dilutions.	16	20	48	28	112
3. Add colonies to bring all treatments to 50 colonies. Did not add enough colonies to the study. An oversight.	17	0	0	1	18
4. The colony totals (add step 2 and step 3). These colonies were streaked thrice to obtain pure cultures.	33	20	48	29	130
5. The colony count after streaking thrice to obtain pure cultures.	50	34	87	48	219
6. Isolates with low quality DNA sequences.	10	8	20	12	50
7. Totals of high quality sequences. Subtracted the low quality DNA sequences from the pure cultures (subtract step 6 from step 5).	40	26	67	36	169
8. All <i>Microbacterium</i> sequences.	17	10	25	19	71
9. Clone <i>Microbacterium</i> sequences.	8	7	17	9	41
10. Totals of <i>Microbacterium</i> sequences. Subtracted the clone sequences from all the <i>Microbacterium</i> sequences (subtract step 9 from step 8).	9	3	8	10	30
11. Totals from subtracting the clone <i>Microbacterium</i> sequences from the high quality sequences (subtract step 9 from step 7).	32	19	50	27	128
12. The total number of isolates that were aligned and included in cladograms.	32	19	50	27	128

Table 16. Rules for selecting the BLAST identification for bacterial endophytes from 10 matches from the fungi database.

Rule 1: Remove all general or broadly named BLAST matches from consideration.

Rule 2: All BLAST matches used for identification will have a percent identity equal to or greater than 97 %.

Rule 3: Select the genus name of the first or first few BLAST matches when the bits scores are the highest of the matches.

Rule 4: Select the genera names of the first BLAST matches when their bits scores are the same, e. g. *Enterobacter/Pantoea*.

Rule 5: Select the family name of the genus or genera when the E-value is low and the percent identities are lower than 97 %.

Table 17. The culturable bacterial endophytes putative identification and classification as described in Bergey's Manual of Systematic Bacteriology 9th edition (Holt et al. 1994). The number of isolates (culturable bacterial endophytes) for each genera are in parenthesis.

Major Category I: Gram-negative eubacteria with cell walls.

Group 4a: microaerophilic straight rods with strictly respiratory metabolism.

Group 5.1: aerobic or facultatively anaerobic straight rods with chemoorganotrophism having respiratory and fermentative metabolism.

Phylum *Bacterioidetes*

Class "*Flavobacteria*"

Genus *Chryseobacterium* (3 isolates)

Phylum *Proteobacteria*

Class "*Alphaproteobacteria*"

Genus *Afipia* (Group 4a, 3 isolates)

Genus *Brevundimonas* (Group 4a, 6 isolates)

Genus *Rhizobium* (Group 4a, 2 isolates)

Genus *Sphingomonas* (Group 4a, 1 isolate)

Class "*Betaproteobacteria*"

Genus *Acidovorax* (Group 4a, 12 isolates)

Class "*Gammaproteobacteria*"

Genus *Pseudomonas* (Group 4a, 8 isolates)

Genus *Stenotrophomonas* (Group 4a, 11 isolates)

Genus *Xanthomonas* (Group 4a, 3 isolates)

Genus *Klebsiella* (Group 5.1, 5 isolates)

Genus *Pantoea* (Group 5.1, 2 isolates)

Informal group *Enterobacter/Pantoea* (Group 5.1, 7 isolates)

Major Category II: Gram-positive eubacteria with cell walls.

Group 17: the cocci

Group 18: the endospore-forming rods and cocci

Group 20: the irregular nonsproing rods

Group 21: the mycobacteria

Group 22: norcardioform actinomycetes, morphologically and culturally similar to the genus *Nocardia*, a bacteria that forms mycelium that can fragment into rod or cocci shaped cells.

Group 23: the actinomycetes with multicellular asexual spores in a multilocular sporangia, a spore case.

Phylum *Firmicutes*

Class "*Bacilli*"

Genus *Staphylococcus* (Group 17, 2 isolates)

Genus *Bacillus* (Group 18, 3 isolates)

Phylum *Actinobacteria*

Class *Actinobacteria*

Genus *Microbacterium* (Group 20, 30 isolates)

Genus *Curtobacterium* (Group 20, 9 isolates)

Genus *Mycobacterium* (Group 21, 2 isolates)

Genus *Amycolatopsis* (Group 22, 2 isolates)

Genus *Geodermatophilus* (Group 23, 1 isolate)

Table 18. The CFUs and distribution of putatively identified culturable bacterial endophytes isolated from surface sterilized crown tissue from four treatments of bermudagrass cultivars, Midlawn and Tifgreen non-infected and Midlawn and Tifgreen infected with *O. herpotricha*.

Putatively Identified Endophytes	Non-Infected		Infected	
	Midlawn	Tifgreen	Midlawn	Tifgreen
<i>Acidovorax</i>	1	1	10	-
<i>Afipia</i>	1	1	-	1
<i>Amycolatopsis</i>	-	-	-	2
<i>Bacillus</i>	3	-	-	-
<i>Brevundimonas</i>	-	4	-	2
<i>Chryseobacterium</i>	2	1	-	-
<i>Curtobacterium</i>	1	7	-	1
<i>Geodermatophilus</i>	-	-	-	1
<i>Klebsiella</i>	5	-	-	-
<i>Microbacterium</i>	9	8	3	10
<i>Mycobacterium</i>	-	2	-	-
<i>Pantoea</i>	-	-	1	1
<i>Pseudomonas</i>	3	5	-	-
<i>Rhizobium</i>	-	1	-	1
<i>Sphingomonas</i>	-	1	-	-
<i>Staphylococcus</i>	-	-	-	2
<i>Stenotrophomonas</i>	5	5	-	1
<i>Xanthomonas</i>	-	3	-	-
Broad Taxa				
<i>Enterobacter/Pantoea</i>	2	5	-	-
<i>Actinobacteria</i>	-	-	-	1
" <i>Alphaproteobacteria</i> "	-	1	4	2
" <i>Betaproteobacteria</i> "	-	2	1	2
<i>Microbacteriaceae</i>	-	1	-	-
<i>Xanthomonadaceae</i>	-	2	-	-
Totals	32	50	19	27

Table 19. Culturable endophytic bacteria *in vitro* antagonism towards the fungal causal agent of Spring Dead Spot, *O. herpotricha*, in bermudagrass grown in different nutrient media. Antagonism was rated on a scale of 0-5 with 0 indicating no antagonism and 5 the highest. MN=Midlawn non-infected, TN=Tifgreen non-infected, MI=Midlawn infected with *O. herpotricha*, TI=Tifgreen infected with *O. herpotricha*, NA=Nutrient Agar, PDA=Potato Dextrose Agar, TSA=Tryptic Soy Agar.

Endophyte Number, Putative Identification	Treat- ment	1X NA	1/5XN A	1X PDA	1/5X PDA	1X TSA	1/5X TSA
219 <i>Bacillus</i>	MI	0	0	0	0	3	0
129 " <i>Betaproteobacteria</i> "	MI	0	0	0	0	3	0
36 <i>Chryseobacterium</i>	MN	0	0	0	0	3	2
20 <i>Enterobacter/Pantoea</i>	TN	2	0	1	0	3	1
22 <i>Enterobacter/Pantoea</i>	TN	3	0	1	1	3	0
24 <i>Enterobacter/Pantoea</i>	TN	1	0	1	1	3	0
37 <i>Enterobacter/Pantoea</i>	MN	1	0	1	0	3	1
44 <i>Enterobacter/Pantoea</i>	MN	1	0	0	0	4	0
215 <i>Klebsiella</i>	MN	3	0	1	0	2	0
78 <i>Microbacterium</i>	TN	1	2	0	0	4	2
60 <i>Pantoea</i>	TI	3	0	1	1	3	0
32 <i>Pseudomonas</i>	TN	1	0	4	0	4	3
33 <i>Pseudomonas</i>	MN	2	0	1	0	4	3
34 <i>Pseudomonas</i>	MN	1	0	4	0	4	3
35 <i>Pseudomonas</i>	MN	2	0	1	0	4	2
48 <i>Pseudomonas</i>	TN	2	2	3	0	4	3
52 <i>Pseudomonas</i>	TN	2	0	4	1	4	3
53 <i>Pseudomonas</i>	TN	2	0	1	0	4	3
38 <i>Stenotrophomonas</i>	MN	2	1	0	0	3	0
40 <i>Stenotrophomonas</i>	MN	2	2	4	0	4	4
41 <i>Stenotrophomonas</i>	MN	1	2	1	0	3	3
42 <i>Stenotrophomonas</i>	MN	2	2	0	0	3	1
46 <i>Stenotrophomonas</i>	TN	3	0	0	0	3	1
49 <i>Stenotrophomonas</i>	TN	2	1	1	0	3	2
51 <i>Stenotrophomonas</i>	TN	3	1	1	1	2	3
57 <i>Stenotrophomonas</i>	TN	2	0	0	0	3	1
59 <i>Stenotrophomonas</i>	TN	2	1	4	1	4	3
477 <i>Stenotrophomonas</i>	TI	0	3	0	0	3	3
50 <i>Xanthomonadaceae</i>	TN	2	1	1	1	3	0
67 <i>Xanthomonadaceae</i>	TN	3	2	0	0	3	0

Table 20. The CFUs of putatively identified culturable bacterial endophytes that displayed antagonism towards *O. herpotricha*. The antagonism ratings were numeric from 0-5, with 5 the highest antagonism response. None of the endophytes assayed displayed the highest rating of antagonism. Only those endophytes with moderate antagonism (3 to 4) are included. The CFUs are listed first followed by the antagonism rating(s) in parenthesis.

Putatively Identified Endophytes	Totals	Non-Infected Midlawn	Tifgreen	Infected Midlawn	Tifgreen
<i>Bacillus</i>	1	1 (3)	-	-	-
<i>Chryseobacterium</i>	1	1 (3)	-	-	-
<i>Klebsiella</i>	1	1 (3)	-	-	-
<i>Microbacterium</i>	1	-	1 (3)	-	-
<i>Pantoea</i>	1	-	-	-	1 (3)
<i>Pseudomonas</i>	7	3 (4, 4, 4,)	4 (4, 4, 4, 4)	-	-
<i>Stenotrophomonas</i>	10	4 (3, 3, 3, 4)	5 (3, 3, 3, 3, 4)	-	1 (3)
Broad Taxa					
<i>Enterobacter/Pantoea</i>	6	2 (3, 4)	3 (3, 3, 3)	1 (3)	-
"Betaproteobacteria"	1	-	-	1 (3)	-
<i>Xanthomonadaceae</i>	2	-	2 (3, 3)	-	-
Totals	31	12	15	2	2

Table 21. Species of culturable bacterial endophytes documented in other studies and isolated from the crown tissue of bermudagrass in this current study that possess antagonism towards the indicated plant pathogen.

Endophyte	Plant and Location	Pathogen	Reference
<i>Bacillus pumilus</i> strain 85	Corn kernel	<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus pumilus</i> strain 85	Corn kernel	<i>Sclerotium rolfsii</i>	Pleban et al. 1995
<i>Enterobacter</i> sp.	Cotton	<i>Fusarium oxysporum</i> f. <i>sp. vasinfectum</i>	Chen et al. 1995
<i>Klebsiella pneumonia</i>	Clover root	<i>Rhizoctonia solani</i>	Struz et al.1998
<i>Klebsiella pneumonia</i>	Potato tuber	<i>Rhizoctonia solani</i>	Struz et al.1998
<i>Microbacterium</i> sp.	Cotton	<i>Fusarium oxysporum</i> f. <i>sp. vasinfectum</i>	Chen et al. 1995
<i>Pantoea agglomerans</i>	Potato tuber	<i>Phytophthora infestans</i> A1, A2	Struz et al. 1999
<i>Pseudomonas fluorescens</i> S3, <i>P. talaasii</i> , <i>P. veronii</i>	Rice	<i>Achyla klebsiana</i>	Adhikari et al. 2001
<i>Pseudomonas fluorescens</i> S3, <i>P. talaasii</i> , <i>P. veronii</i>	Rice	<i>Pythium spinosum</i>	Adhikari et al. 2001
<i>Stenotrophomonas maltophilia</i> C3	Grass foliage	<i>Rhizoctonia solani</i>	Giesler and Yuen 1998

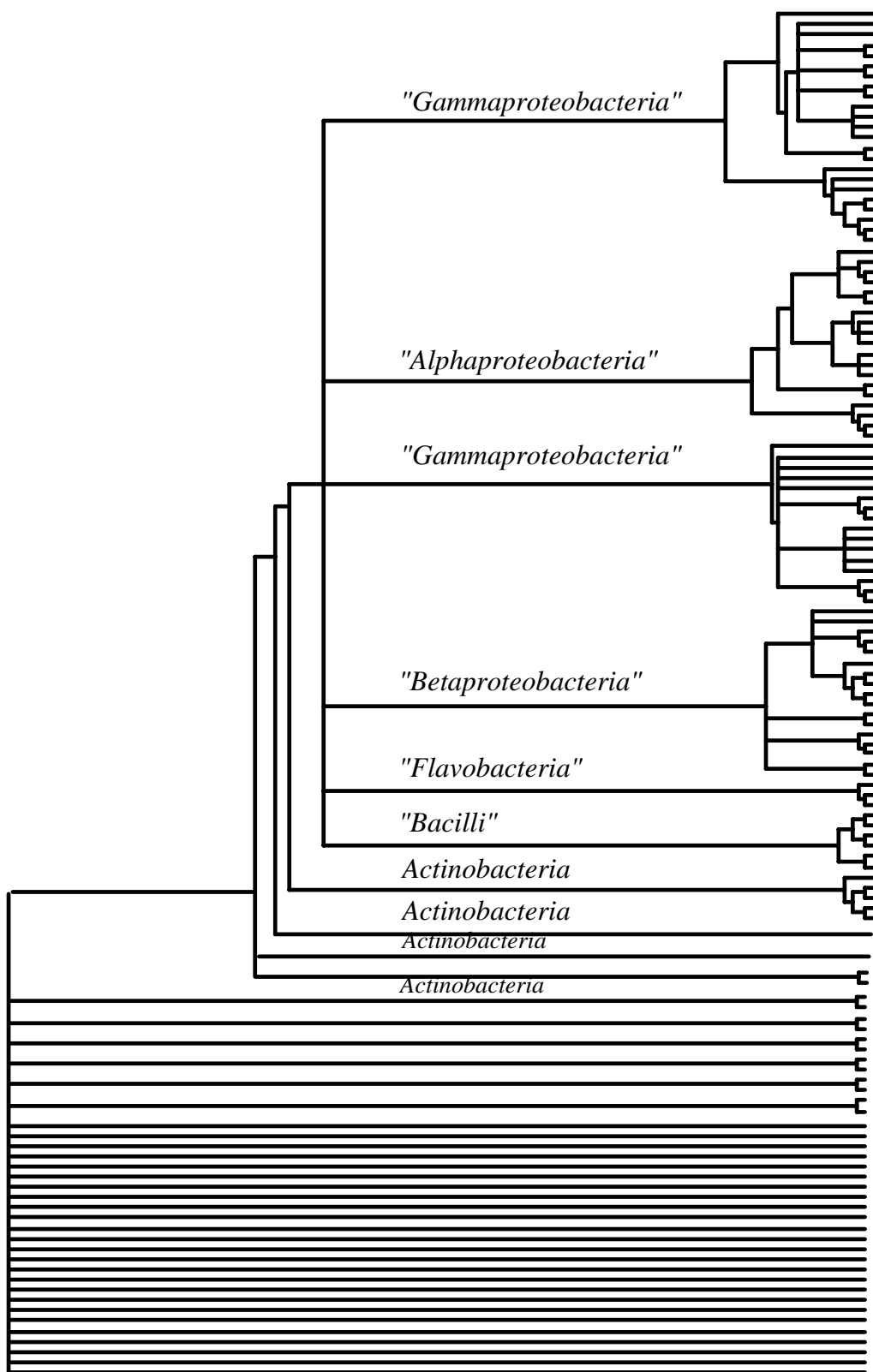
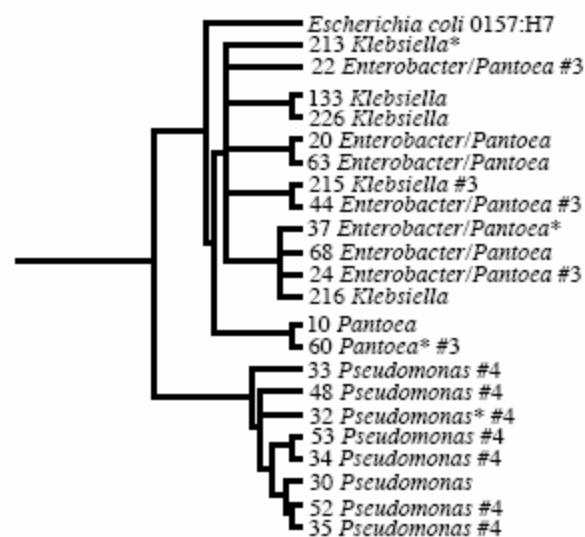
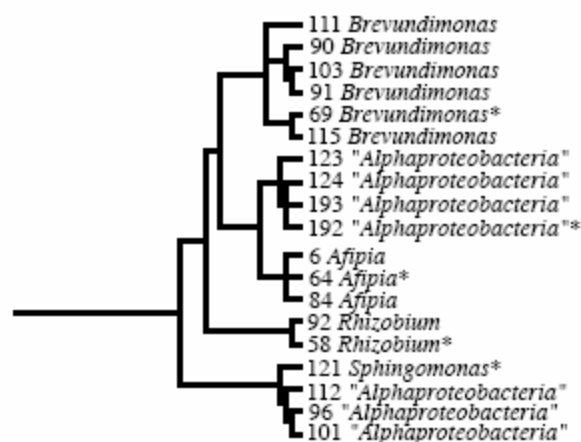


Figure 1. Neighbor-joining bootstrapped (1000 X) cladogram, bootstrap values less than or equal to 500 collapsed, of the culturable bacterial endophytes with the addition of *Bacillus megaterium* and *Escherichia coli* 0157:H7.



"Gammaproteobacteria" I



"Alphaproteobacteria"

Figure 2. The "Gammaproteobacteria" I and "Alphaproteobacteria" clades from Figure 1. The asterisk indicates the clade representative sequences. The number sign denotes those endophytes with *in vitro* antagonism towards *O. herpotricha*. The following number is the antagonism rating on a scale of 0-5 with 0 indicating no antagonism and 5 the highest.

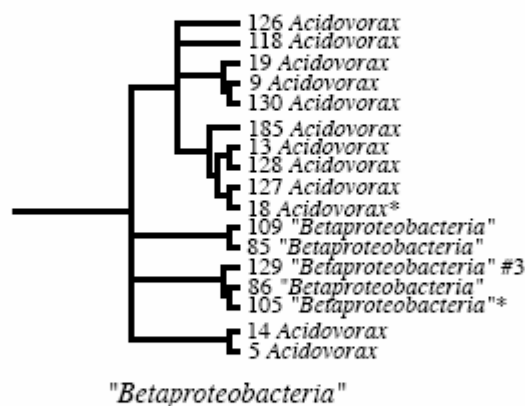
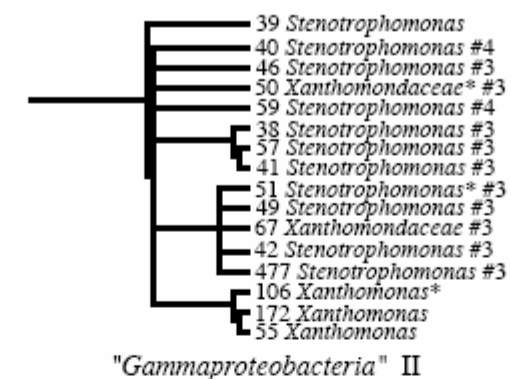


Figure 3. The "Gammaproteobacteria" II and "Betaproteobacteria" clades from Figure 1. The asterisk indicates the clade representative sequences. The number sign denotes those endophytes with *in vitro* antagonism towards *O. herpotricha*. The following number is the antagonism rating on a scale of 0-5 with 0 indicating no antagonism and 5 the highest.

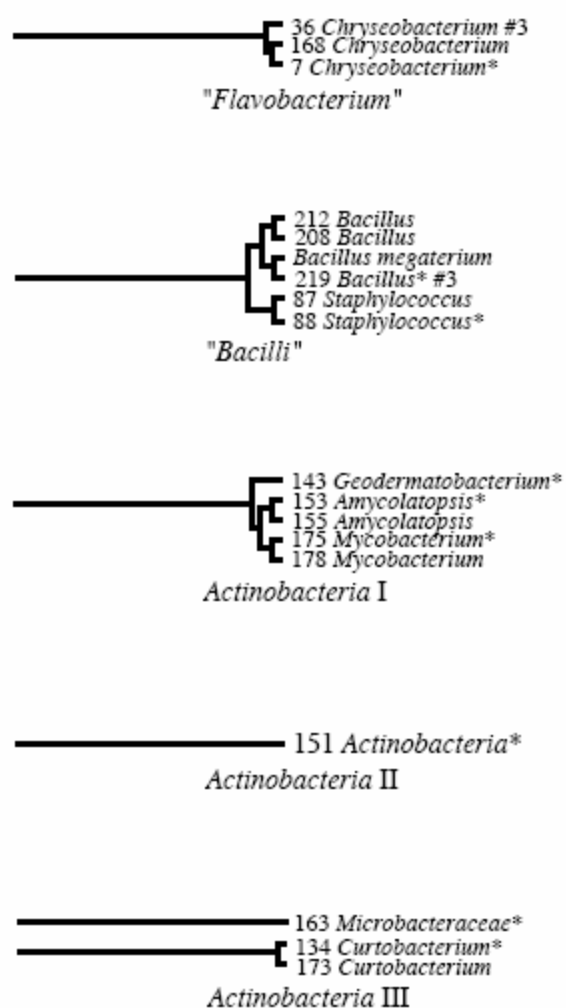
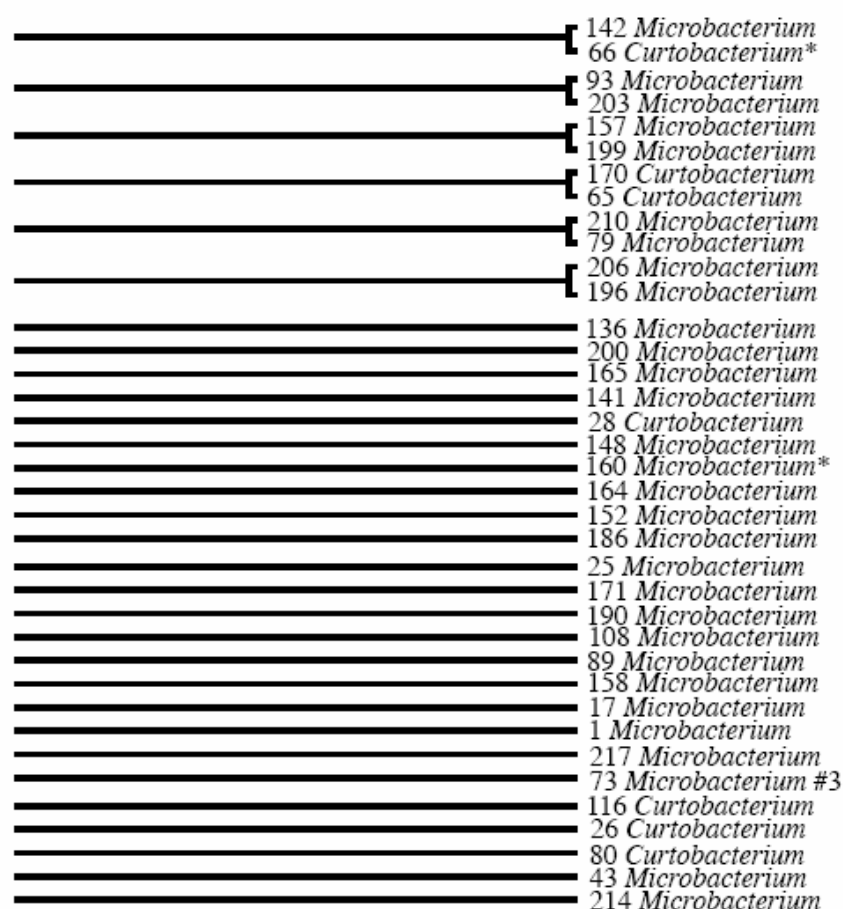


Figure 4. The "Flavobacteria", "Bacilli", and Actinobacteria I, II, and III clades from Figure 1. The asterisk indicates the clade representative sequences. The number sign denotes those endophytes with *in vitro* antagonism towards *O. herpotricha*. The following number is the antagonism rating on a scale of 0-5 with 0 indicating no antagonism and 5 the highest.



Actinobacteria IV

Figure 5. The *Actinobacteria IV* clade from Figure 1. The asterisk indicates the clade representative sequences. The number sign denotes the endophyte with *in vitro* antagonism towards *O. herpotricha*. The following number is the antagonism rating on a scale of 0-5 with 0 indicating no antagonism and 5 the highest.

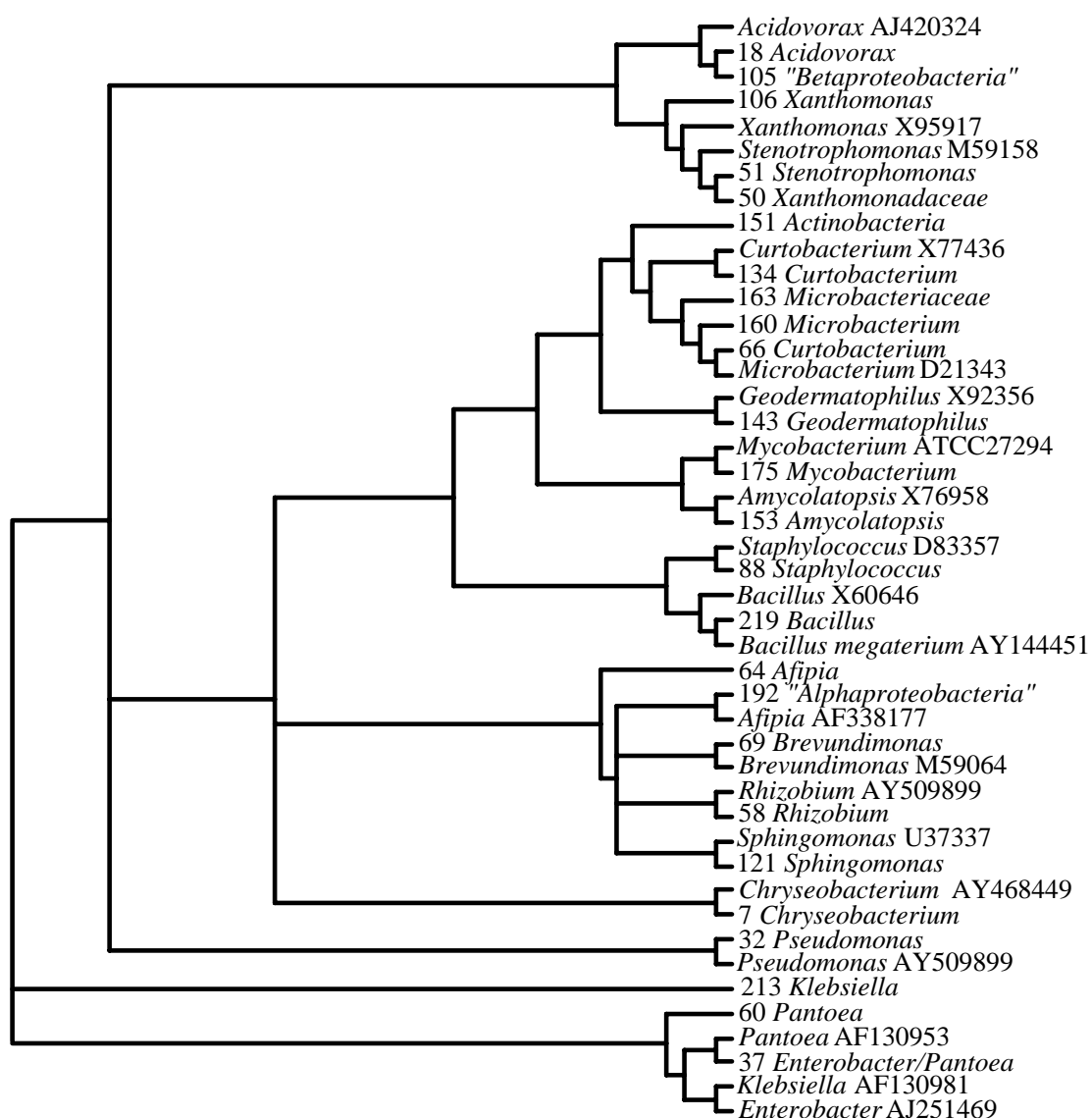


Figure 6. Neighbor-joining bootstrapped (1000 X) cladogram containing 16S rDNA sequences, 25 endophyte representatives (number, genus), *Bacillus megaterium*, and 19 type species 16S rDNA sequences. The type species name is followed by NCBI accession numbers, single and double letter prefixes or American Type Culture Collection number with the prefix ATCC.

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DEVELOPMENT AND APPLICATION OF REAL-TIME PCR ASSAY FOR *O. HERPOTRICA* USING TAQMAN[®] CHEMISTRY

ABSTRACT

Spring dead spot (SDS) is a destructive fungal disease of bermudagrass. SDS is caused by three closely related *Ophiosphaerella* species, *O. korrae*, *O. herpotricha* and *O. narmari*. A real-time PCR assay with TaqMan[®] chemistry was developed to detect absolute quantities of *O. herpotricha* DNA in plant and soil samples from 8 SDS infected cultivars varying in resistance to SDS. Turf plugs were removed from the edge of the dead spot in November 2001 from dead spots visualized the previous spring. There were 2 of 24 plant samples from SDS-susceptible cultivars Pyramid and Princess with detectable levels of *O. herpotricha* DNA at 0.3 and 21.0 ng DNA g⁻¹, respectively. The pathogen was detected in 19 of 23 soil samples, with a range of 0.1 to 5.6 ng of *O. herpotricha* DNA g⁻¹ soil. There was no obvious relationship between resistance and susceptibility of the cultivars and the levels of *O. herpotricha* DNA in plant and soil samples from the eight cultivars. The spatial distribution of *O. herpotricha* in infected SDS resistant Midlawn and susceptible Greg Norman-1 cultivars of bermudagrass was also measured. The turf plug samples were removed from the center and edge of the dead spot and at 20 and 41 cm from the edge of the dead spot in November 2001 based on spot location visualized the previous spring. The spatial distribution was patchy for both cultivars in plant and soil samples. The highest plant levels of *O. herpotricha* DNA

were 390 and 680 ng *O. herpotricha* DNA g⁻¹ plant material for Greg Norman-1 and Midlawn, respectively. The highest soil levels of *O. herpotricha* DNA were 4.6 and 9.8 ng g⁻¹ for Greg Norman-1 and Midlawn, respectively. There were no clear relationship between resistance of a cultivar and quantity of DNA.

INTRODUCTION

Developing a control system for plant diseases depends on understanding the factors that influence pathogen infection, plant resistance to pathogens, and pathogen distribution. Central to each of these areas is the knowledge of the presence and abundance of pathogens of interest. Until recently, researchers had limited capability to identify and quantify individual pathogens. The advent of polymerase chain reaction (PCR) techniques allowed for the sensitive detection of specific pathogens and semi-quantitative assessment of pathogen abundance. However, PCR based measurements rely on post-PCR processing steps that are time-consuming, difficult to automate, and may only be semi-quantitative at best. The development of real-time PCR methods during the last decade allows for automated, truly quantitative, analysis of pathogen DNA following each PCR amplification cycle. These advances provide for rapid, sensitive, and accurate quantification of pathogen DNA. However, this recent approach to pathogen measurement has yet to be applied in studies of fungal infections of turfgrasses. Spring dead spot (SDS), caused by three closely related species of *Ophiosphaerella* Spegazzini 1909 (*Ascomycota*), is the most destructive fungal disease of bermudagrass (Tisserat et al. 1989, Duble 1996; Watschke et al. 1995). In the United States, SDS is a

major disease in the transition climatic zone: the northern zone of bermudagrass adaptation, where autumn and winter temperatures induce bermudagrass dormancy (Wetzel III et al. 1999; Fermanian et al. 2003). SDS infects 3-6 year old highly managed turf, such as golf course fairways and greens, athletic fields, and residential lawns. SDS fungi form black plaques on root surfaces entering the root in advanced infections and eventually killing the plant. SDS is thought to be most active in the spring and fall when temperatures favor the fungal growth (Fermanian et al. 2003). Furthermore, In the fall as temperatures are lowered, infection leaves the plant weakened and more susceptible to winter kill. As the temperatures increase in the spring bermudagrass resumes its growth and the damage becomes readily apparent in the form of unsightly straw-colored, slightly depressed, round patches of turf, ranging from a few cm to about 1 m in diameter (Baird et al. 1998; Tisserat et al. 2004).

The distribution of the *Ophiosphaerella* sp. in the USA depends on geographic location. *O. herpotricha* (Fr.:Fr.) J. C. Walker infects bermudagrass in Kansas, Oklahoma, and Texas (Tisserat et al. 1989; Tisserat et al. 1994). *O. korrae* (J. C. Walker & A. M. Smith) Shoemaker & C. E. Babcock infects bermudagrass in California (Endo et al. 1985), Maryland (Crahay et al. 1988), North Carolina, Kentucky (Tisserat et al. 1994), and Australia (Tisserat et al. 1991). *O. narmari* Wetzel, Hulbert & Tisserat infects bermudagrass in Australia, New Zealand (Wetzel III et al. 1999), and California (Tisserat et al. 2003). These fungal species can be distinguished by the length of their ascospores, *O. herpotricha* the longest, *O. korrae* intermediate, and *O. narmari* the shortest. Visual identifications are difficult because the ascocarps are not easily induced in nature or in artificial media (Tisserat et al. 1994). Correct identification of the infecting SDS species

is critical because treatment may be species dependent (Tisserat et al. 2003).

Conventional PCR has been used as a diagnostic method for all three *Ophiosphaerella* spp. (Tisserat et al. 1994) and has also been used to quantify other phytopathogenic fungi (Cullen et al. 2002), but results are often unreliable (Schena et al. 2004). PCR is a cyclic reaction that is repeated many times resulting in a tremendous amplification of the target DNA. Near the end of the last cycle in the PCR, reagents become exhausted (Bohm et al. 1999) halting the reaction. For this reason, attempts to quantify the PCR product in the later cycles may underestimate the amount of target DNA. To quantify the PCR product, conventional PCR amplifications are usually followed by agarose gel electrophoresis and staining with ethidium bromide or various hybridization/blotting methods and capture techniques. These post-PCR procedures are error prone, time consuming, labor intensive, and impractical to automate, and they generate environmental wastes (Chen et al. 1997; Oberst et al. 1998; Bohm et al. 1999; Zhang and Yuen 1999).

A real-time quantitative PCR with TaqMan[®] probes was developed about 10 years ago, providing rapid, sensitive, and accurate quantification of target DNA. Early reports of real-time quantitative PCR with TaqMan[®] chemistry include the detection of the food-borne bacterial human pathogens *Listeria monocytogenes* (Bassler et al. 1995), *Salmonella* (Chen et al. 1997), and *Escherichia coli* Migula 1895 O157:H7 (Oberst et al. 1998). Some of the first applications in plant pathology include real-time PCR assays of pathogens in plant tissues including the *potato leafroll virus* (Schoen et al. 1996), the fungi *Diaporthe phaseolorum* (Cook & Ellis) Sacc. 1882, and *Phomopsis longicolla*

(Zhang and Yuen 1999). These early successes led to more recent applications of TaqMan[®] methods including measurements of phytopathological fungi in both plant and soil, quantifying *Helminthosporium solani* Durien & Mont. 1849 (Cullen et al. 2001), *Rhizoctonia solani* J. G. Kuhn 1858 AG-3 (Lees et al. 2002), and *Colletotrichum coccodes* (Wallr.) S. Hughes 1958 (Cullen et al. 2002). To date, there has been no assay to quantify *Ophiosphaerella* sp. DNA in the natural environment. This study is the first to develop real-time PCR for quantifying the causal agent of spring dead spot, *O. herpotricha*.

The technology of real-time PCR with TaqMan[®] probes is based on the use of PCR forward and reverse primers for DNA amplification and a fluorescent probe complementary to a specific internal sequence of the target DNA for specific detection. The 5' end of the probe is labeled with a fluorescent reporter dye and the 3' end is labeled with a quencher. When the probe is in the free state in solution or hybridized to the amplified DNA, little if any fluorescence is generated due to the close proximity of the quencher dye to the fluorescent moiety. During PCR both forward and reverse primers anneal to their priming sites and the TaqMan[®] probe anneals internally within the target DNA. During the PCR extension phase, while DNA is being copied and as the polymerase contacts the internally annealed TaqMan[®] probe, the 5'-exonuclease activity of the *Taq* polymerase releases the 5' fluorescent dye from hybridized probes. The released dye is able to fluoresce upon excitation because it is no longer in close proximity to the quencher dye. Upon excitation, the real time PCR Thermal Cycler detects and records the accumulation of fluorescence in the sample after every cycle (Mumford et al.

2000; Winton et al. 2002; Mayer et al. 2003). In addition, the TaqMan[®] probe exhibits a high degree of specificity for hybridizing to the target DNA. The probe is so specific that it will not detect DNA sequences differing from the target by a single nucleotide (Schna et al. 2004). The real-time PCR assay is extremely sensitive and can detect a single copy of target DNA in specific systems (Zhang and Yuen 1999). As many as 384 samples can be run at a single time, in as little as 3 hours, making it one of the highest throughput systems available for detecting and quantifying nucleic acids. Furthermore, selective quantification of target DNA is available at the end of the real-time PCR assay, eliminating the need for time consuming post-PCR methods (Zhang and Yuen 1999; Cullen et al. 2002; Atkins et al. 2003).

There are two methods to quantify the amplification of DNA in sample extracts, relative and absolute quantification. Relative quantification analyzes changes in gene expression in a given sample relative to a reference sample. Relative quantification is useful if one is most interested in comparing DNA concentration among experimental treatments. Absolute quantification measures amplified DNA by interpolating the amplicon quantity from a standard curve generated from DNA standards of known concentration (Applied Biosystems 2005). Absolute quantification is required if one is interested not only in the relative amounts among experimental treatments, but also the concentration of DNA. Absolute quantification is essential if one is interested in determining DNA concentrations over an extended time period where it would be difficult to run all samples at a given time.

In this report, we describe the development of a real-time PCR assay for the absolute quantification of *O. herpovirica* DNA. The objectives of our study were aimed

to: (1) optimize a real-time quantitative PCR assay with TaqMan[®] probe/primer set to quantify *O. herpotricha* in bermudagrass plant tissue and soil samples, (2) use this assay to document the spatial distribution of *O. herpotricha* infection, and (3) document the relationship between resistant and susceptible cultivars of bermudagrass and SDS.

MATERIALS AND METHODS

All bermudagrass plots used in this study were located at the Oklahoma State University Turfgrass Research Center, Stillwater, Oklahoma under the direction of Dr. Dennis Martin, Department of Horticulture and Landscape Architecture, Oklahoma State University. Samples of turf were removed November 21, 2001 from 2 to 6 PM using a plug cutter, extracting a core 2.5 cm diameter X 6 cm long. Each individual plug location was chosen from a site characterized for infection with *O. herpotricha* during the previous spring. The plug cutter was washed in fresh water and scrubbed with a bottle brush to remove all plant and soil material before the next sampling. The harvested plugs were placed separately into individually labeled zip-lock plastic bags stored on ice, transported back to the lab, and stored at 4 °C for further processing.

Sample Harvesting for Measuring Spatial Distribution of *O. herpotricha*

Eighteen to 22 bermudagrass plugs were harvested from each of three plots of the resistant cultivar Midlawn and the highly susceptible cultivar Greg Norman-1 (GN-1) plots (Figs. 7-12). Plugs were cut from the center, periphery, and 20 and 41 cm from the periphery of the dead spot. Unequal sampling from each of these locations occurred as an oversight. Plugs from GN-1 Plots B and C and Midlawn Plots A and C were arranged in a radial pattern as illustrated (Figs. 8-10, 12). However, sampling had to be altered

because some of the dead spots were too close together. This occurred in Midlawn Plot B (Fig. 11) and GN-1 Plot A, where dead spots overlapped significantly (Fig. 7).

Sample Harvesting for 8 Cultivars of Bermudagrass

Bermudagrass cultivars OKC 19-9, ‘Patriot’ (OKC 18-4) [*Cynodon dactylon* L. (Pers.) X *C. transvaalensis* Burt-Davies], Tifway, Numex Sahara, Mirage, Sydney (SW 1-7), Pyramid, and Princess were selected to represent a wide range in resistance and susceptibility to *O. herpotricha*. For each cultivar a total of 12 plugs, four plugs from the periphery of the dead spot in each of three plots, were collected in November whose location was determined by the location of the dead spot in the previous spring:.

Total Plant DNA Isolation

The plant component of each plug was separated from the soil component manually, and the soil component was reserved for total soil DNA extraction. The plant material was placed in a plastic weigh-boat and scrubbed gently in several changes of Nanopure water (Barnstead, Dubuke, IA) with a toothbrush until the water was clean, approximately 4 changes of water. A clean weigh-boat was used for each plug. The crown tissue, rhizomes, and stolons were excised from the roots and stems using an 70 % ethanol washed razor blade. The crown tissue, rhizomes, and stolons were combined and placed into a 1.7 mL or 15 mL centrifuge tube depending on the mass of tissue. The tubes were sealed with two layers of Parafilm[®] and capped, frozen at –20 ° C for two hours, then lyophilized overnight (Labconco, Kansas City, MO). The tubes were stored at –20 ° C until processed further. The plant material was ground under liquid nitrogen to a fine powder using a mortar and pestle, and the DNA was isolated using the DNeasy[®]

Plant Mini Kit (Qiagen Inc., Valencia, CA) following manufacturer's instructions. The isolated DNA was stored at -20°C .

Total Soil DNA Isolation

In the laboratory, roots were carefully removed from the soil plug and the soil saved in 1.7 mL or 15 mL centrifuge tubes. The tubes were sealed with two layers of Parafilm[®] and frozen at -20°C for 2 hours, then lyophilized overnight and stored at -80°C . Total soil DNA was isolated using the UltraClean[™] Soil DNA Isolation Kit (MoBio Inc., Sunnyvale, CA) following manufacturer's instructions for maximum yields. The isolated DNA was stored at -20°C .

O. herpotricha Genomic DNA Isolation

Using sterile technique, *O. herpotricha* hyphae growing on nutrient agar (Sigma, St. Louis, MO) were scraped into a 500 mL flask containing with 250 mL of nutrient broth (Becton Dickinson, Cockeysville, MD) and shaken at 150 rpm for seven days at room temperature. Under these conditions the fungus hyphae formed a spherical matt which were recovered by filtration through a Buchner funnel lined with a paper filter. The hyphae were transferred to 15 mL centrifuge tubes, sealed with two layers of Parafilm[®], frozen at -20°C for 2 hours, and then lyophilized overnight. The lyophilized hyphae were ground under liquid nitrogen and stored in 1.7 mL microcentrifuge tubes at -80°C . The DNeasy[®] Plant Mini Kit was used to isolate genomic DNA from *O. herpotricha* following manufacturer's instructions using 23 mg of ground lyophilized *O. herpotricha* hyphae per extraction.

O. herpotricha Primers and TaqMan[®] Probe

The primers and TaqMan[®] probe were designed to amplify and detect *O. herpotricha* DNA of the internal transcribed spacer (ITS) region (Tisserat et al. 1994), and were designed from a series of primers generated by the Primer Express Version 1.0 software (Applied Biosystems, Foster City, CA). The software generates a series of primer/probe combinations and ranks them according to a penalty score following certain guidelines for optimizing the TaqMan[®] probe sequence. The DNA sequence should have a T_m of 68 – 70 °C to ensure the probe hybridization to the complementary target prior to polymerase extension. The 5' end of the probe should be as close to the 3' end of the primer without overlapping to ensure immediate displacement and cleavage of the fluorescent dye by the polymerase. Guanine should not be at the 5' end of the probe sequence because guanine quenches the fluorescence of the fluorescent dye. Also runs of 4 or more guanines should be avoided because of possible excessive fluorescence quenching (Applied Biosystems 2002; Bustin and Nolan 2004).

Primers were synthesized by the Nucleic Acids Core at the Pennsylvania State University, University Park, PA. The forward primer was 5' TGA ACC TGC GGA AGG ATC A3', 19 bases long, with a T_m of 59 °C, and % GC of 53. The reverse primer was 5' GTA ATA GAC ATA ACC CGT CTG CGT AG 3', 26 bases long with a T_m of 58 °C and % GC of 46. The TaqMan[®] probe (Biosearch Technologies, Inc., Novato, CA) sequence was 5' 6-FAM d(ACA CGA TAG TAC AGG CCC CAA GTG TAG AAC AA)BHQ-1 3', 32 bases long with a T_m of 68 °C and % GC of 47. The reporter dye, 6-FAM (6-carboxyfluorescein), has a maximum excitation wavelength of 494 nm (± 5 nm). The quencher dye is a black hole quencher, BHQ-1 (4-methyl-2-nitrobenzylazo-

2'-methyl-5'-nitrobenzylazo-4''-N,N-di(2-hydroxyethyl) azobenzene), with a maximum quenching wavelength of 534 nm (± 5 nm). The PCR amplicon was 80 bases long with a T_m of 78 °C, and % GC of 46.

Real-Time PCR Master Mix and Real-Time PCR Cycle

The real-time PCR master mix for each 100 μ L reaction was composed of 25.0 μ L of Universal Master Mix (ABI PN# 4304437, or Eurogentec RT-QP2X-03WOU), 2.0 μ L of primer 1450 for a final concentration of 400 nM, 2.0 μ L of primer 1451 for a final concentration of 400 nM, 10.0 μ L of TaqMan[®] probe for a final concentration of 200 nM, 6.0 μ L of water, and 5.0 μ L of plant or soil extract.

The real-time PCR light cycler (ABI Prism 7700 sequence detector, Applied Biosystems, Foster City, CA) at the Nucleic Acids Core of the Pennsylvania State University was programmed as follows: Stage One at two min at 50 °C; Stage Two at ten min at 95 °C to hold; then Stage Three at 45 cycles at 15 sec at 95 °C, one min at 60 °C; followed by a final hold at two min at 25 °C. Stage One digests the uracil-N-glycosylase. Stage Two denatures uracil-N-glycosylase and activates the Ampli-taq Gold DNA polymerase. Stage Three amplifies the amplicon (Lees et al. 2002). Sample fluorescence was measured for each sample after stage three of each cycle.

Standard Curve for Real-Time PCR

A serial dilution was made from a stock solution of 31 ng of *O. herpotricha* genomic DNA μ L⁻¹. The final concentrations of the standards were obtained by diluting the stock concentration ten fold in a series of five steps (from 3.1 ng μ L⁻¹, to 0.00031 ng μ L⁻¹). A standard curve of *O. herpotricha* DNA was generated by amplifying 5.0 μ L of each dilution in 95 μ L of Master Mix. The dilution series was run three times and the

values were averaged. All five standards were included in one 96-well plate per day of analyses.

Analyses of Real-Time PCR Data

The ABI Prism 7700 software (Applied Biosystems, Foster City, CA) recorded the fluorescence of each reaction for every PCR cycle, created a real-time amplification plot, calculated threshold cycle (C_t) values, and generated a standard curve graph. The C_t value is a preset value that is near the front end of the linear range of the real-time PCR response curve. Absolute quantitation of *O. herpotricha* DNA was calculated based on the regression equation derived from the standard curve of C_t values vs. absolute amount of *O. herpotricha* DNA.

Optimization of *O. herpotricha* Amplification in Plant and Soil Samples

The *O. herpotricha* primers, TaqMan[®] probe, and light cycler parameters were optimized using DNA extracts from 4 GN-1 plugs removed from the periphery of the dead spot because these samples had the highest probability of being infected with *O. herpotricha*. A series of 10 fold dilutions was prepared for each of the plant and soil DNA extracts. The addition of polyvinylpyrrolidone (PVP-40), 8 % final volume, was added to half of the samples and was found to be necessary for generating good amplification signals.

Specificity of the *O. herpotricha* Primers and TaqMan[®] Probe Set

Genomic DNA isolated from *Rhizoctonia solani* and *Pythium arrhenomanes* were tested against the *O. herpotricha* primers and TaqMan[®] probe set. A negative control, Buffer AP1 from the DNeasy[®] Plant Mini Kit (Qiagen Inc., Valencia, CA), and a positive

control, *O. herpotricha* genomic DNA, were included in the same 96-well plate as the *R. solani* and *P. arrhenomanes* samples.

Statistical Analyses

Statistical analyses of *O. herpotricha* DNA concentrations in extracts of plant and soil samples were performed in collaboration with Professor Mark Payton of the Department of Statistics, Oklahoma State University, Stillwater, OK. DNA concentrations were transformed using a logarithm transform, and ANOVA was performed using PROC MIXED option of SAS software (SAS 2001). A multiple comparison was performed by looking under differences in least square means.

RESULTS

O. herpotricha Primers and TaqMan[®] Probe Set

The sequence similarity of the forward and reverse primers and the TaqMan[®] probe for detection of *O. herpotricha* were evaluated individually by basic local alignment search tool (BLAST) alignment (Altschul et al. 1997) against the National Center for Biotechnology Information (NCBI) sequence database. The nucleotide sequence of the forward primer matched accessions from *O. herpotricha* (U04861) and *O. korrae* (U04862) (Tisserat et al. 1994) perfectly (Table 22) as well as those of 20 other genera of fungi, including: *Coprinus* Pers. 1797 (AY461840 (Keirle et al. 2003)), *Corpinopsis* (AY461833 (Keirle et al. 2003)), and *Inonotus* P. Karst. 1879 (AY436626 (Yun et al. 2003)). The reverse primer showed 100% similarity in all 26 base pair (bp) positions to four *O. herpotricha* strains, including: U04861, AF101797, AF101796, and

AF101795 (Wetzel III et al. 1998). The NCBI BLAST match for the 31 bp TaqMan[®] probe DNA sequence showed a 100% match with only one strain of *O. herpotricha* (U04861) and a single base pair deviation with two other strains of *O. herpotricha* (AF101795 and AF101798 (Wetzel III et al. 1998)). A single match with 2 base pair deviation was observed with *O. namari* (AF101803 (Wetzel III et al. 1998)).

Experimental Verification of Selectivity

The specificity of the real-time PCR assay was evaluated by analyzing genomic DNA extracted from *Pythium arrhenomenes* (Oomycota) and *Rhizoctonia solani* (Basidiomycota) two fungal species widely divergent from *Ophiosphaerella* sp. (Ascomycota). Duplicate analyses of reaction mixtures containing either 62 ng of *R. solani* DNA or 145 ng of *P. arrhenomenes* DNA failed to give threshold fluorescence after 45 amplification cycles.

Sensitivity and Dynamic Range of the Real-Time PCR Assay

Sensitive quantification of *O. herpotricha* DNA was achieved using the forward and reverse primers in conjunction with the TaqMan[®] probe described above. For each day plant and soil samples were assayed, DNA standard solutions ranging in concentration from 3.1 ng to 310 fg of *O. herpotricha* DNA μL^{-1} were analyzed in duplicate to construct a standard curve (Fig. 13). The assay performance varied slightly, (relative standard deviation (RSD) = 7 %), day to day, and easily detected the lowest concentration (310 fg) *O. herpotricha* DNA standard spanning range of four orders of magnitude. The least-squares best fit of $\log[\text{DNA}]$ vs. cycle number for threshold detection gave correlation coefficients ranging from $R^2=0.921$ to 0.997.

Optimization for Matrix Effects

Initial measurements performed on extracts of GN-1 plant samples from two plugs collected from the periphery of the dead spot yielded positive detection of *O. herpotricha* in both plant samples (Table 23). Further experiments were conducted to optimize the PCR reaction by diluting the plant and soil samples or by adding the phenolic scavenger PVP to the extract. In addition, we spiked the plant and soil samples with known amounts of DNA to observe the effect of additional DNA on the amplification process. As expected, diluting the plant samples 10 fold increased the Ct values by 6.7 cycles in non-PVP treated and 4.5 cycles in the PVP treated tissues. Addition of PVP to the plant samples yielded an increase of 4.4 cycles in full strength samples and 2.2 cycles in the 10 fold diluted samples. Spiking the real-time PCR amplification mixture with a relatively large amount of *O. herpotricha* DNA (1 ng) resulted in an expected and dramatic reduction in numbers of cycles from an average of 38 cycles to 21.5 cycles. Neither PVP nor dilution was employed in subsequent plant samples real time PCR measurements because the optimization experiment without PVP and dilution amplified *O. herpotricha* DNA.

When the same initial experiments were performed on soil samples no pathogen DNA was detected. To test our hypothesis that soil extracts might contain inhibitory substances, the soil extracts were spiked with 1 ng of *O. herpotricha* DNA and PVP, 8 % final volume, and analyzed again (Table 23). In contrast to the results from plants, no pathogen DNA was detected in spiked soil extracts at full strength (1X). Dilution of soil extracts and the addition of PVP reduced the inhibition in real-time PCR samples from unknown inhibitory substances. Diluting the spiked soil DNA extracts 10- and 100-fold

resulted in dramatic reduction in average cycle numbers when compared to undiluted extracts from 44.5 to 23.2. These results for spiked DNA samples were similar to those obtained from equivalent concentrations of *O. herpovirica* DNA in buffer (data not shown). Soil extracts diluted 10 and 100 fold showed no detectable levels of pathogen DNA. In subsequent real-time PCR assays, soil samples were conservatively diluted 100-fold and PVP, 8 % final volume, was added.

Sample to Sample Variation

The reproducibility of the real-time assay was high for both plant and soil samples (n=2). Replicate analyses were compared for a total of 149 plant and 147 soil assays yielding an average standard deviation of 0.29 and 0.54 C_t, respectively.

Use of Assay – 8 Cultivar Study

To test the real-time PCR assay, field samples were assayed for *O. herpovirica* DNA in three triplicate pooled plant and soil samples from eight bermudagrass cultivars (Table 24). The samples were collected around the periphery of the infection zone. The pathogen was detectable in only one plant extract from each of two most susceptible cultivars, Princess and Pyramid, with 21 and 0.3 ng of *O. herpovirica* DNA gram⁻¹ plant material, respectively. The most susceptible cultivars were the only ones to show any detectable levels of pathogen DNA with this technique. The levels of detection were well above the background level of the assay. The pathogen was detected in 19 of 23 soil extracts ranging as high as 5.6 ng of *O. herpovirica* DNA gram⁻¹ soil in replicate 2 of Tifway and as low as 0.1 ng in rep 3 of Princess and rep 1 of Numex Sahara. In contrast to the plant DNA, the results showed little relationship to cultivar susceptibility. The

highest soil values were found in cultivar Tifway and the lowest with Patriot soil extracts. Every cultivar gave at least one positive soil assay result.

Spatial Distribution of *O. herpotricha* in Spots with Susceptible Greg Norman-1

The real-time PCR assay was used to document the spatial distribution of *O. herpotricha* in plant and soil samples for susceptible GN-1 cultivars. Samples were obtained from infection zones during the spring when spots were visible. Sampling was done in the center of the spot where the turfgrass had died, the periphery where the grass was thinned, and at two locations successively more distant from the periphery. Real-time PCR assays were performed on both soil and plant materials. Higher average readings of *O. herpotricha* were found in the plant material than in the soil. Although there were no statistically significant differences among the various geographical locations in the plant material, there was a trend in that direction ($p=0.11$). The lack of statistically defined differences reflects wide fluctuations in readings from sample to sample. Standard deviations of the data ranged from 2.8-1.2 times the absolute value of the average value itself. Despite the lack of statistically discernable differences, average DNA concentrations in the plant material tended to be higher in the center and the periphery than away from the periphery. On average, decreasing levels occurred in plant material from 20 and 41 cm from the periphery. The highest levels were found in the center of the dead spot in Plot A at 390 ng g⁻¹ sample. Plot A had much greater average levels of *O. herpotricha* DNA than either Plot B or C. Only three samples from Plot B showed any detectable levels of *O. herpotricha* DNA.

Readings for the soil extracts from the GN-1 were all very low with numerous non-detects. There were no significant differences ($p= 0.482$) among the different

locations due to the wide degree of variability and low readings of the fungus in the soil. In contrast to the plant extracts, the soil extracts from GN-1 Plot B were all below the limits of detection (Table 25). The soil extracts from GN-1 Plots A and C contained generally the same levels of *O. herpotricha* DNA in the four sampling sites ranging from non-detects to 4.6 ng *O. herpotricha* DNA gram⁻¹ soil.

Spatial Distribution of *O. herpotricha* in Spots with Midlawn

The real-time PCR assay was used to document the spatial distribution of *O. herpotricha* in plant and soil samples for resistant Midlawn cultivars (Table 26). In contrast to Greg Norman-1, Midlawn plant samples showed much lower real-time PCR readings. However, the differences between cultivars were not significantly different. Midlawn plant extracts from Plot A had readings of *O. herpotricha* DNA slightly above the limits of detection (Table 26) in all but 6 samples. Only 2 positives were found in Plot B and one in Plot C. The one positive in Plot C was the highest level found in all plant and soil samples collected in this study, at 680 ng DNA g⁻¹. There were no significant differences among the means with respect to location in plant samples in the Midlawn plots.

The readings of *O. herpotricha* DNA in the Midlawn soil extracts ranged from non-detects to 8.9 ng of *O. herpotricha* DNA gram⁻¹. The level of *O. herpotricha* DNA was higher in the periphery in the soil extracts from the Midlawn plots than from the other locations. This coincides with the Greg Norman-1 plant tissues but not in soil samples, nor in the Midlawn plant samples.

DISCUSSION

Goals of This Study

The goals of this study were to (i) develop a real-time quantitative PCR assay using TaqMan[®] chemistry to quantify the soil phytopathogen *O. herpotricha* in plant and soil samples, (ii) use this assay to compare levels of *O. herpotricha* DNA in crown tissue of resistant and susceptible cultivars of bermudagrass and in associated soil, and (iii) document the spatial distribution of SDS in crown tissue and soil for two cultivars of bermudagrass, resistant Midlawn and susceptible Greg Norman-1. The performance of the assay was evaluated based on its specificity for detection of *O. herpotricha* DNA against two unrelated fungal genera, the ability of the assay to quantify low levels of DNA from this pathogen in plant and soil matrices, and the dynamic range of the assay response over a wide range of DNA concentrations.

The real-time quantitative PCR assay using TaqMan[®] chemistry and absolute measurements was developed to detect *O. herpotricha* DNA in plant and soil samples with a limit of detection of 31 fg *O. herpotricha* DNA g⁻¹ sample. Four strains of *O. herpotricha* and one strain of *O. narmari* can be theoretically detected by the assay. *R. solani* and *P. arrhenomanes* genomic DNA was not detected by the real-time PCR assay. This assay exhibits the sensitivity and selectivity necessary for its application in studies of *O. herpotricha* infections.

Assay Selectivity

The *O. herpotricha* primers and TaqMan[®] probe were selected from the ITS rDNA sequence of *O. herpotricha* strain (Tisserat et al. 1994). The ITS region was used because it is a species specific hypervariable region and there are over 100 copies per haploid genome in many fungi (Tisserat et al. 1994; Bohm et al. 1999; Atkins et al. 2003), allowing for potential detection of very low levels of *O. herpotricha* compared to assays detecting single copy sequences per haploid genome, e. g. β -tubulin gene (Winton et al. 2002). Based on the forward and reverse primers alone, the real-time PCR assay will be selective for four strains of *O. herpotricha* (U04861, AF101795, AF101796, and AF101797). There is a possibility the assay will detect one strain of *O. narmari* (AF101798) because the reverse primer may initiate amplification and the TaqMan[®] probe will base pair with the amplicon (Table 22). This possibility needs to be experimentally examined. Based on the theoretical specificity of the TaqMan[®] probe, other related fungi including *O. korrae* and *Leptospharella korrae* (syn = *O. korrae*) differ from the TaqMan[®] probe sequence in two or three bases and all other database entries gave yet poorer matches. The selectivity of the TaqMan[®] probe sequence will not allow annealing to DNA sequences with 2 or more mismatched bases (Schena et al. 2004). We anticipate our TaqMan[®] probe will not detect non-*O. herpotricha* organisms, except *O. narmari* (AF101798). The combined theoretical specificity of the forward and reverse primers with the TaqMan[®] probe should make this assay specific for only one fungal species, *O. herpotricha*.

The specificity of the *O. herpotricha* assay was tested against *Pythium arrhenomenes* and *Rhizoctonia solani* genomic DNA, two species that are widely divergent from the *Ophiosphaerella* sp. During the real-time PCR analyses, fluorescence did not rise above the threshold levels, indicating the *O. herpotricha* assay did not detect *P. arrhenomenes* and *R. solani* DNA. Access to pure cultures of other fungal species was limited, therefore only 2 phytopathogenic fungi were evaluated using this assay. In the future, closely related fungal strains should be tested with this assay to determine the specificity. However, recent reports of other real-time PCR assays of microbial DNA using TaqMan[®] chemistry have demonstrated selective detection of target sequences (Olivira et al. 2002; Mayer et al. 2003; Cullen et al. 2001) when tested against numerous fungal or bacterial species. The selectivity of the TaqMan[®] assay developed in the current study is expected to be high based on the 2001 report from Merck Research Laboratories that 1000-fold discrimination was obtained for sequences differing by a single nucleotide (Bleicher et al. 2001).

Limits of Detection and Dynamic Range for *O. herpotricha* Genomic DNA

The dynamic range, the span of the standard curve between the highest and lowest concentration of *O. herpotricha* DNA, and limits of detection were determined by measuring the number of cycles needed to achieve threshold fluorescence for serial dilutions of *O. herpotricha* genomic DNA. The linear range of the *O. herpotricha* DNA standard curve spans at least 4 orders of magnitude, from 31 fg to 3.1 ng of *O. herpotricha* DNA μL^{-1} . The dynamic range is comparable to that in assays developed for *Glomus mosseae* (T. H. Nicolson & Gerd.) Gerd. & Trappe 1974, *Phytophthora citricola* Sawada 1927, *P. infestans* (Bohm et al. 1999), *Diaporthe phaseolorum*, *Phomopsis*

longicolla Hobbs 1985 (Zhang and Yuen 1999), and *Phaeocryptopus gaeumannii* (T. Rohde) Petr. (Winton et al. 2002).

The limits of detection were 1.4, 3.9, and 26.3 fg of *O. herpotricha* DNA μL^{-1} for three separate but identical real-time PCR assays, as determined from three standard curves generated on three different days when optimization and experimental samples were assayed. These assays were performed in the same laboratory with the same light cycler. Samples that gave a mean of 42 C_t almost always had one replicate that did not reach threshold fluorescence, and were considered below the limits of detection.

Interference by Sample Matrix, Plant and Soil Samples

The challenges in developing this particular assay lie in optimizing conditions for analysis and determining limits of detection in plant and soil samples. The plant and soil DNA extracts were tested to determine if the real-time PCR assay would amplify *O. herpotricha* DNA. The full strength plant extract matrix did not inhibit DNA amplification compared to plant extracts spiked with 1 ng of *O. herpotricha* DNA μL^{-1} and non-spiked extracts (Table 23) suggesting that inhibitory compounds may not be present in the plant extracts. PVP occasionally had a small inhibitory effect on DNA amplification when comparing the change in C_t for plant and soil samples with and without PVP to real-time PCR blanks. However, the full strength soil extract matrix inhibited DNA amplification for both *O. herpotricha* spiked and non-spiked extracts. Diluting the soil extract dramatically lowered the number of cycles in an assay indicating there may be some inhibitory substances in the soil matrix. Subsequent soil assays at 1:10 and 1:100 dilutions, with and without PVP 8% final volume, were equally successful in DNA amplification. These results led us to chose, conservatively, a 1:100

dilution with PVP, 8% final volume, to ensure successful amplification of the remaining soil samples, even though the 1:10 dilution without PVP was sufficient for the test soil sample. The optimization process was easy, quick, and simple and requires minimal laboratory skill.

For analysis of plant and soil samples, the *O. herpotricha* real-time PCR assay limit of detection was always less than 30 fg μL^{-1} as determined by extrapolating the standard curve to a C_t value of 42 cycles. A C_t value of 42 cycles was determined by subtracting the minimum C_t from the maximum C_t for a given sample extract and plotting against C_t . Forty-two C_t corresponded to the lowest DNA concentration that could reliably be detected in both plant and soil replicates. For comparison, the limit of detection for a *Rhizoctonia solani* AG-3 real-time quantitative PCR assay was 168 fg of *R. solani* AG-3 DNA parts per billion (Lees et al. 2002).

Ease of Use

The real-time quantitative PCR with TaqMan[®] probes is easy to perform. Grinding of plant material under liquid nitrogen is the most time consuming step in sample processing. One set of 42 samples can be prepared by one individual during an 8-hour period. Furthermore, DNA isolation from plant and soil samples was simplified by using commercial kits. The kit for soil DNA extraction does not require special sample vials nor a bead beater; only a vortexer and centrifuge are necessary. Real-time PCR assays require about 3 hours of cycle time, approximately half the time of conventional PCR assays, not including post-amplification processing.

Assay Reproducibility

The reproducibility of the assay was determined by comparing values of replicate analyses (n=2) for 149 plant and for 147 soil samples. The plant samples had a smaller standard deviation average, 0.29 real-time PCR cycle (C_t), than the soil samples, 0.54 C_t . The deviations between replicate measurements using the real-time PCR assays are less than one cycle. Previous real-time quantitative PCR studies have established that duplicate assays are a sufficient number of replications for the quantification of target DNA (Lees et al. 2002; Winton et al. 2002).

Spatial Distribution of the Pathogen in Soil and Plant Crown Tissue for 8 Cultivars

Real-time PCR with TaqMan[®] chemistry was used to determine if levels of *O. herpotricha* infection correlated with the resistance and susceptibility of 8 cultivars of bermudagrass. In this study, according to Dr. Dennis Martin's unpublished data, the most resistant cultivar was OKC19-9 followed by Patriot, Mirage, Tifway, Sydney, Numex Sahara, Pyramid, and Princess. Other studies show similar but not identical disease ratings for these cultivars (Morris 2002). *O. herpotricha* was detected in 19 of 23 soil samples and in 2 of 24 plant samples (n=3).

Even though there were measurable levels of *O. herpotricha* in most soil samples, the crown tissue of bermudagrasses did not display disease symptoms during the November collection of turf plugs. This finding is in agreement with the small number of positives in plant material. It has been proposed that *O. herpotricha* hyphae are dormant during summer and winter, but actively colonize bermudagrass during spring and autumn when soil temperatures range from 10-25 °C (Fermanian et al. 2003). Our observations and measurements are inconsistent with extensive pathogen colonization of plant tissues

at the time turf plugs were removed from the turf plot. Future studies might resolve the timing of pathogen colonization of plant tissues by applying real-time PCR to measure *O. herpotricha* levels throughout the year.

Spatial Distribution of the Pathogen in Plots of Resistant Midlawn and Susceptible Greg Norman-1 Cultivars

We documented the spatial distribution of *O. herpotricha* in plant and soil samples of 2 infected cultivars of bermudagrass, susceptible GN-1 and resistant Midlawn (n=3) to demonstrate the feasibility of using the real-time PCR assay in this and similar epidemiological studies.

The GN-1 Plot A had the highest overall levels of *O. herpotricha* DNA of all the GN-1 and Midlawn plots. This coincides with the fact that the GN-1 Plot A had three widely overlapping dead spots in April 2001, and had the highest visible infection area relative to all other plots. The high levels of pathogen in GN-1 Plot A plant extracts stand in contrast to those in all other cultivars and plots. Either the pathogen is extensively colonizing these plants during autumn, or the spring infection has persisted through the dormant summer season.

The Midlawn plant extracts contained lower readings of *O. herpotricha* than those of GN-1 plant extracts though the readings were not significantly different from each other. The soil readings did not vary greatly between the two cultivars. Midlawn is resistant to *O. herpotricha* and this may explain the lower readings of *O. herpotricha* in Midlawn plant extracts compared to GN-1 plant extracts. GN-1 is susceptible to infection but there is no evidence to support that different cultivars support different soil levels of *O. herpotricha*. Further experimentation is necessary to resolve this issue.

The concentrations of *O. herpotricha* DNA from all samples revealed a patchy distribution in all three GN-1 and Midlawn plots. Up until now, little was known about the distribution of *Ophiosphaerella* spp. in soils throughout the season. Extrapolation from behavior of other soil fungi may not shed light onto the behavior of *Ophiosphaerella* spp either. Harris et al. (2003) found *Rhizoctonia solani* hyphae increased in density with increasing bulk density of soil, though they did not study any other soil parameters. Goodman and Trofymow (1998) found the abundance of ectomycorrhizae in mature and old-growth stands of Douglas-fir was related to soil chemistry. The distribution of fungi in southern Ohio hardwood forest soils was related to long-term moisture patterns in the soil and soil texture (Morris and Boerner 1999). However, Frey et al. (1999) found the fungal biomass in conventional and no-tillage agroecosystems along two climatic gradients was not strongly influenced by soil texture, pH, aggregation, organic C and N levels, or climate gradient effects, but positively related to soil moisture. These studies emphasize the need to determine the microscale patterns and the biotic and abiotic influences on fungal distribution for individual or small groupings of fungi.

The extreme variability found in this study of real-time PCR detectable DNA of *O. herpotricha* suggests an alternative sampling strategy. Many more samples than the number used in this study are necessary to make statistically relevant comparisons among treatments. Since the variation may be due to the patchy distribution of the fungus in the plots, it may be necessary to collect many more samples and analyze the prevalence of the DNA in terms of frequency of PCR positives. This would enable one to use standard PCR and simple agarose gel electrophoresis. On the other hand the use of real-time PCR

in such a system will allow quantitation of *O. herpotricha* DNA for each treatment, adding additional information for statistical comparisons.

CONCLUSION

We have developed a standard-curve real-time quantitative PCR assay with TaqMan[®] chemistry to identify and quantify the DNA levels of *O. herpotricha* in plant and soil samples. Theoretically the real-time PCR assay can detect 4 strains of *O. herpotricha* and one strain of *O. narmari*. The assay could not detect *R. solani* nor *P. arrhenomenes* genomic DNA. The plant total DNA extract was assayed directly and the soil total DNA extract needed dilution to 100 X and the addition of PVP. This assay is quantitative, sensitive, selective, rapid, and easy to perform. This powerful assay, which facilitates assessment of fungal prevalence, distribution and diversity, will be useful in the study of other plant diseases.

Table 22. Nucleotide sequences of the ITS region (Tisserat et al. 1994) showing (A) positions of the primers and TaqMan[®] probe within the *O. herpotricha* U04861 sequence, and BLAST alignments with the most closely related database entries for (B) the forward primer, (C) the reverse primer complement, and (D) the TaqMan[®] probe.

(A)

Forward primer	TaqMan [®] probe
5' gtagg <u>tgaacctgcggaaggatca</u> tt <u>acacgatagtagcaggccccaagtgtagaacaa</u>	
Reverse primer	
<u>actacgcagacgggttatgtctattac</u> ccttg 3'	

(B)

Forward primer BLAST Match

Database Entry	DNA Sequence (5' to 3' + strand)
<i>O. herpotricha</i> (U04861)¶	tgaacctgcggaaggatca
<i>O. korrae</i> (U04862)	tgaacctgcggaaggatca

¶ This sequence was used as the forward primer

(C)

Reverse primer BLAST match

Database Entry	DNA Sequence (5' to 3' + strand)
<i>O. herpotricha</i> (U04861)§	ctacgcagacgggttatgtctattac
<i>O. herpotricha</i> (AF101795)	ctacgcagacgggttatgtctattac
<i>O. narmari</i> (AF101798)	<u>actatgc</u> gacgggttatgtctattac
<i>O. narmari</i> (AF101803)	cta <u>tgcgg</u> acgggcctatgtctattac
<i>O. korrae</i> (U04862)	<u>actcatggg</u> cgggttatgtctattac
<i>O. korrae</i> (AF101792)	ct <u>gtatggg</u> cgggttatgtctattac
<i>L. korrae</i> (AF86626)	ct <u>gtatgggt</u> gggttatgtctattac
	* *** *****

§ This sequence was used to produce the reverse primer

(D)

TaqMan[®] probe BLAST match

Database Entry	DNA Sequence (5' to 3' + strand)
TaqMan [®] probe	<u>acacgatagtagcaggccccaagtgtagaacaa</u>
<i>O. herpotricha</i> (U04861)#	acacgatagtagcaggccccaagtgtagaacaa
<i>O. herpotricha</i> (AF101795)	-cagcatagtagcaggccccaagtgtagaacaa
<i>O. narmari</i> (AF101798)	-cacgatagtagcaggccccaagtgtagaacaa
<i>O. narmari</i> (AF101803)	-cacgatagtagcaggccccaag <u>cg</u> tagaacaa
<i>O. korrae</i> (U04862)	acacgatagtagcaggccccaagtgcagcaciaa
<i>O. korrae</i> (AF101792)	-cacgatagtagcaggccccaagtgcagcaciaa
<i>L. korrae</i> (AF486626)	acacgatagtagcaggccccaagtgcagcaciaa
#TaqMan probe sequence	***** ***** * ** *****

Table 23. Mean threshold cycle values (C_t) and total DNA quantities from duplicate real-time quantitative PCR measurements of *O. herpotricha* DNA extracted from Greg Norman-1 Plot A plant and soil samples with and without spikes of 1 ng *O. herpotricha* DNA. Values demonstrate the extent of assay inhibition by matrix constituents and the effects of extract dilution and addition of 0.8% polyvinylpyrrolidone (PVP) to PCR reaction mixtures. ND = not detectable.

Plant Samples

	<u>Mean C_t (no spike)</u>	<u>Measured DNA in extract (ng)</u>	<u>Mean C_t (1 ng DNA spike)</u>	<u>Measured DNA in spiked extract (ng)</u>
Description				
Plant extract 1X, no PVP	33.0	9.7×10^{-4}	20.9	9.5×10^{-1}
Plant extract 1X, with PVP	37.4	7.7×10^{-5}	21.5	6.8×10^{-1}
Plant extract 0.1X, no PVP	39.7	2.1×10^{-5}	21.8	5.7×10^{-1}
Plant extract 0.1X, with PVP	41.9	5.8×10^{-6}	21.7	6.1×10^{-1}

Soil Samples

	<u>Mean C_t (no spike)</u>	<u>Measured DNA in extract (ng)</u>	<u>Mean C_t (1 ng DNA spike)</u>	<u>Measured DNA in spiked extract (ng)</u>
Description				
Soil extract 1X, no PVP	44.0	ND	45.0	ND
Soil extract 1X, with PVP	44.0	ND	44.0	ND
Soil extract 0.1X, no PVP	45.0	ND	23.1	2.7×10^{-1}
Soil extract 0.1X, with PVP	44.0	ND	23.1	2.7×10^{-1}
Soil extract 0.01X, no PVP	45.0	ND	23.0	2.9×10^{-1}
Soil extract 0.01X, with PVP	44.0	ND	23.8	1.8×10^{-1}

Table 24. *O. herpotricha* DNA concentrations (ng DNA g⁻¹ sample) for plant and soil samples from 8 cultivars differing in resistance to infection. Values are means of duplicate determinations.

Cultivar	Field Spot Area* <i>cm</i> ²	Rep 1		Rep 2		Rep 3		Average	
		Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
		<i>ng DNA</i>							
Princess	2995	0.0	1.3	0.0	1.2	21.0	0.1	7.0	0.9
Pyramid	2132	0.0	0.3	0.3	1.7	0.0	0.0	0.1	0.7
Numex Sahara	1589	0.0	1.0	0.0	0.0	0.0	2.8	0.0	1.3
Sydney (SW1-7)	1578	0.0	0.1	0.0	1.7	0.0	1.6	0.0	1.1
Tifway	1452	0.0	0.8	0.0	5.6	0.0	2.4	0.0	2.9
Mirage	1432	0.0	1.0	0.0	1.7	0.0	0.2	0.0	1.0
Patriot	632	0.0	0.4	0.0	0.0	0.0	0.5	0.0	0.3
OKC 19-9	210	0.0	#	0.0	2.2	0.0	0.0	0.0	1.1

* Field data collected by Dr. Dennis Martin of the OSU Horticulture Department from 2000 to 2002. The higher the number the more susceptible the cultivar.

Sample was lost during storage, and not analyzed.

Table 25. *O. herpotricha* DNA concentrations (ng DNA g⁻¹ sample) for Midlawn plant and soil samples. Values are means of duplicate C_t determinations. 0.0 = non-detect. - = no sample. *O. herpotricha* DNA in the periphery was significantly different from other means (p=0.017)

Location	A Plot		B Plot		C Plot		Average± Stdev	
	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
Center	ng DNA g ⁻¹ sample							
	0.1	0.3	0.1	0.1	0.0	0.3	0.1 ± 0.05	0.9 ± 1.24
	0.1	2.7	-	-	-	-		
Center Plot Averages	0.1	1.5	0.1	0.1	0.0	0.3	56.7 ± 1.96	1.6 ± 2.60
Periphery	0.1	0.3	0.1	0.1	0.0	8.9		
	0.1	0.9	0.0	0.1	680	3.0		
	0.2	3.7	0.0	0.1	0.0	0.1		
	0.1	1.8	0.0	0.1	0.0	0.5		
	0.1	1.7	0.0	0.1	170.0	3.1		
Periphery Plot Averages							0.0 ± 0.04	0.1 ± 0.34
20 cm from Periphery	0.1	0.1	0.0	0.1	0.0	0.0		
	0.1	0.1	0.0	0.0	0.0	0.0		
	0.0	0.1	0.0	0.0	0.0	0.0		
	0.0	0.1	0.0	0.1	0.0	0.0		
	0.0	0.1	0.0	0.1	0.0	0.0		
	0.1	0.1	0.0	0.1	0.0	0.0		
	0.1	0.1	0.0	0.1	0.0	0.0		
	0.1	0.1	0.0	1.7	0.0	0.0		
			-	-	-	-		
20 cm Plot Averages	0.1	0.1	0.0	0.3	0.0	0.0		
41 cm for Periphery	0.1	0.1	0.0	0.0	0.0	0.0	0.0 ± 0.04	0.0 ± 0.05
	0.0	0.1	0.0	0.0	0.0	0.0		
	0.1	0.1	-	-	0.0	0.1		
	0.0	0.1	-	-	0.0	0.1		
	0.1	0.1	-	-	0.0	0.0		
	0.1	0.1	0.0		0.0	0.0		
	0.1	0.1	0.0	0.0	0.0	0.0		
	0.0	0.1	0.0	0.0	0.0	0.0		
41 cm Plot Averages	0.1	0.1	0.0	0.0	0.0	0.0		
Overall Plot Averages	0.1	0.5	0.0	0.2	32.4	0.6	14.2	0.7

Table 26. *O. herpotricha* DNA concentrations (ng DNA g⁻¹ sample) for Greg Norman-1 plant and soil samples. Values are means of duplicate C_t determinations. 0.0 = non-detect, - = no sample. There were no statistically observable differences among locations or plots.

Location	A Plot		B Plot		C Plot		Average ± Stdev	
	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
Center	ng DNA g ⁻¹ sample							
	7.4	0.2	0.0	0.0	7.9	0.5	67.6 ± 158	0.8 ± 1.2
	390.0	0.9	0.0	0.0	0.0	3.2		
Center Plot Averages	198.7	0.6	0.0	0.0	4.0	1.9	92.5 ± 119	0.8 ± 1.3
Periphery	270.0	0.1	0.0	0.0	0.1	0.7		
	180.0	0.4	0.3	0.0	0.0	0.3		
	240.0	1.2	0.0	0.0	150.0	1.0		
	270.0	4.6	0.0	0.0	0.0	1.2		
Periphery Plot Averages	240.0	1.6	0.1	0.0	37.5	0.8	17.4 ± 49	0.3 ± 0.5
20 cm from Periphery	0.1	0.3	0.0	0.0	0.1	0.1		
	0.1	0.0	0.0	0.0	0.1	0.1		
	36.0	0.1	0.0	0.0	5.7	1.0		
	6.7	0.0	0.0	0.0	0.0	0.7		
	-	-	0.0	0.0	0.2	0.1		
	12.0	1.5	0.1	0.0	0.1	1.3		
	170.0	0.0	0.0	0.0	0.1	1.2		
	0.0	0.0	0.0	0.0	0.1	0.9		
	170.0	0.0	-	-	-	-		
20 cm Plot Averages	49.4	0.2	0.0	0.0	0.9	0.7		
41 cm for Periphery	-	-	0.0	0.0	0.1	0.1	7.4 ± 21	0.4 ± 0.8
	0.0	0.0	21.0	0.0	0.1	0.7		
	0.0	0.0	0.0	0.0	0.1	1.6		
	84.0	2.0	0.0	0.0	0.2	3.0		
	-	-	4.3	0.0	0.1	0.1		
	0.1	0.0	0.0	0.0	0.1	0.1		
	0.0	0.1	0.0	0.0	0.1	0.1		
	53.0	0.0	0.0	0.0	0.1	0.1		
41 cm Plot Averages	22.9	0.4	3.2	0.0	0.1	0.7		
Overall Plot Averages	94.5	0.6	1.2	0.0	7.9	0.8	46.2	0.6

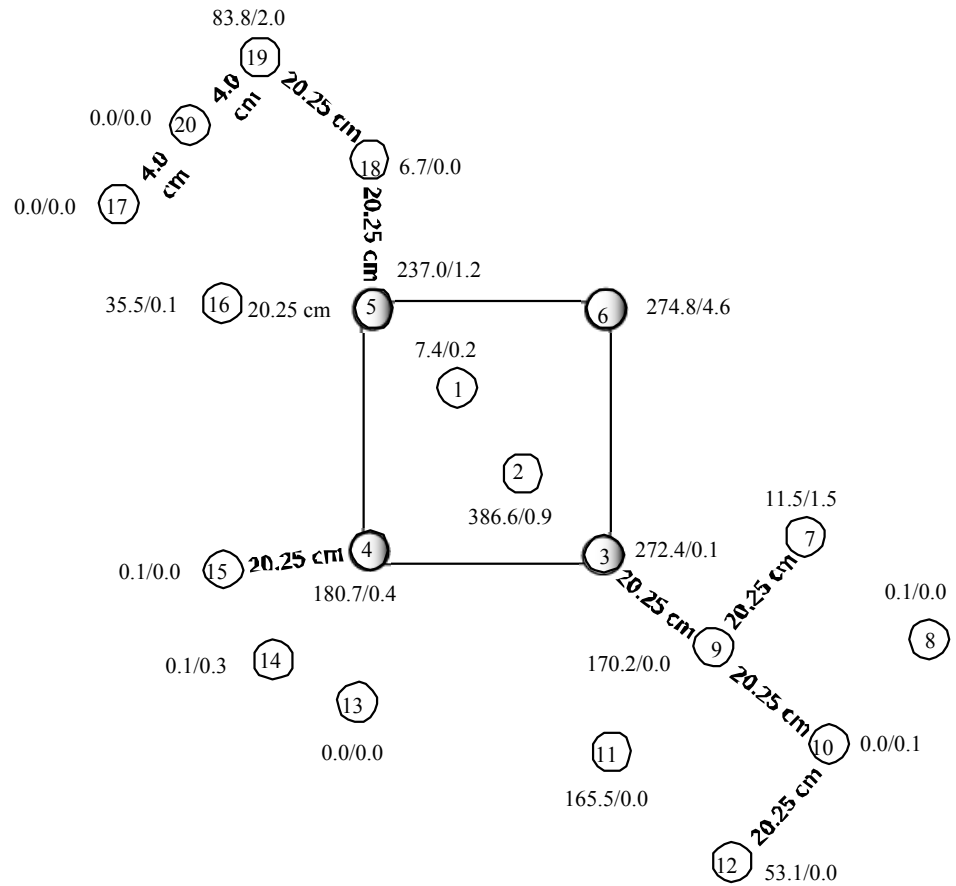


Figure 7. The location of 2.5 cm diameter plugs in the Greg Norman-1 Plot A. The dead spot is indicated by the square and measured 24.25 cm North to South and 22.25 cm West to East. North is at the top. The three dead spots in Plot A merged into one another, hence the odd shape of the dead spot and the unusual placement of the plugs. The plugs radiated from the edge of the dead spot and other plugs in 20.25 cm increments, except plugs 17, 20, and 19. The values are plant/soil ng of *O. herpotricha* DNA/g sample.

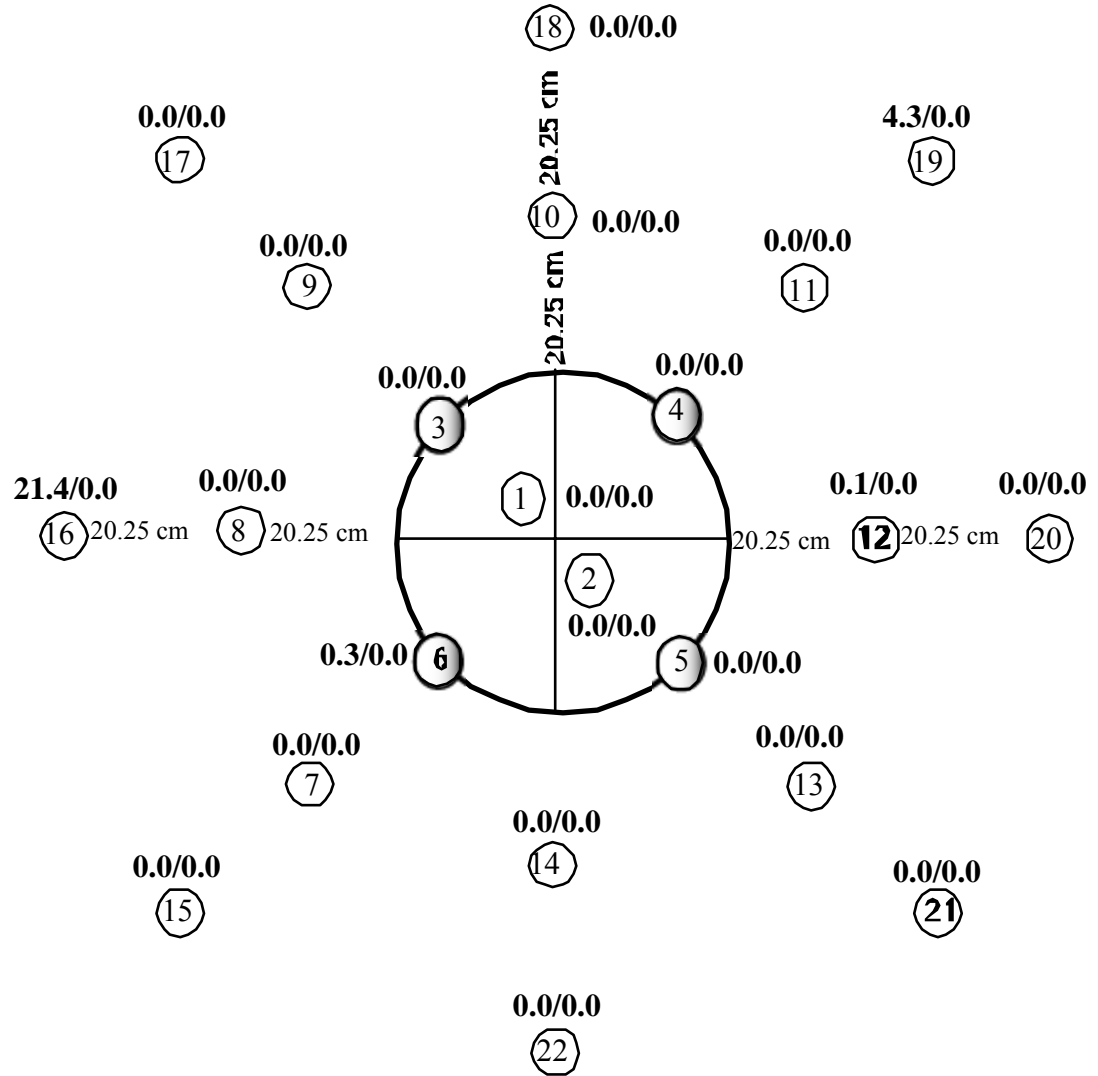


Figure 8. The location of 2.5 cm plugs in the Greg Norman-1 Plot B. The dead spot is indicated by the circle and measured 19.0 cm North to South and 22.25 cm West to East. North is at the top. The plugs radiated from the edge of the dead spot in 20.25 cm increments. The values are plant/soil ng of *O. herpotricha* DNA/g sample.

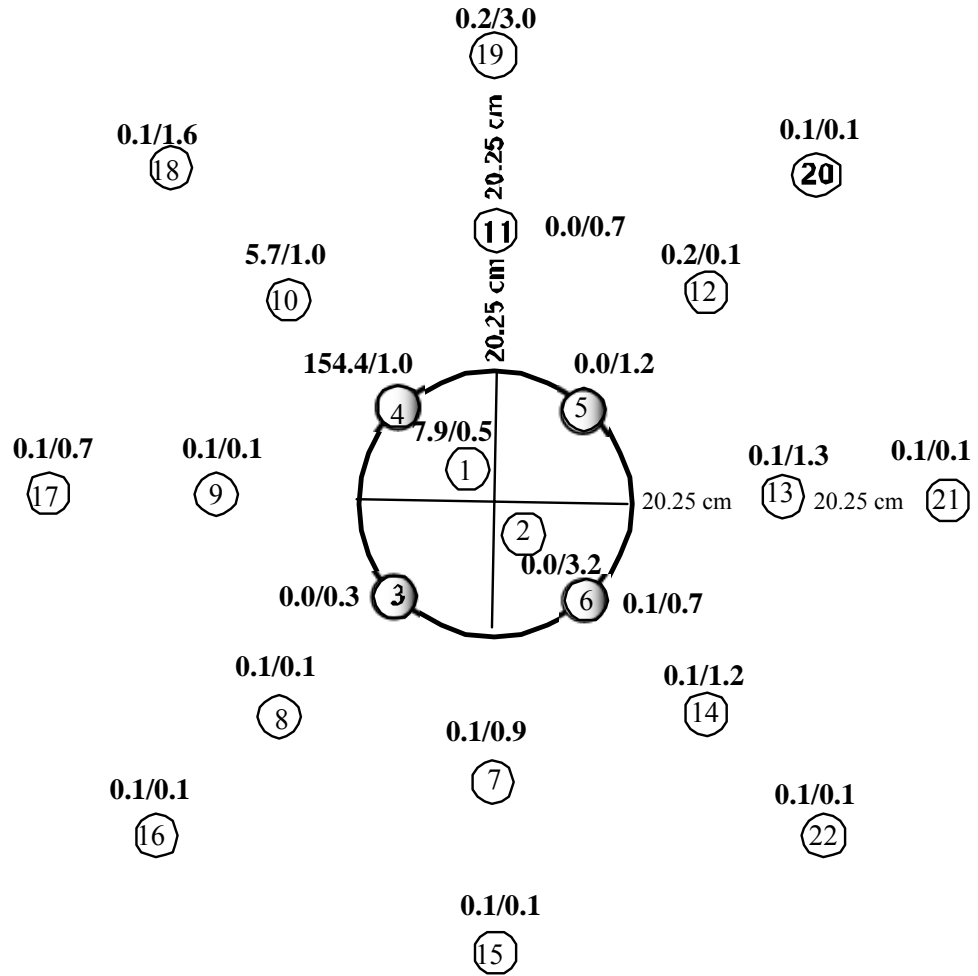


Figure 9. The location of 2.5 cm plugs in the Greg Norman-1 Plot C. The dead spot is indicated by the circle and measured 20.5 cm North to South and 20.0 cm West to East. North is at the top. The plugs radiated from the edge of the dead spot in 20.25 cm increments. The values are plant/soil ng of *O. herpotricha* DNA/g sample.

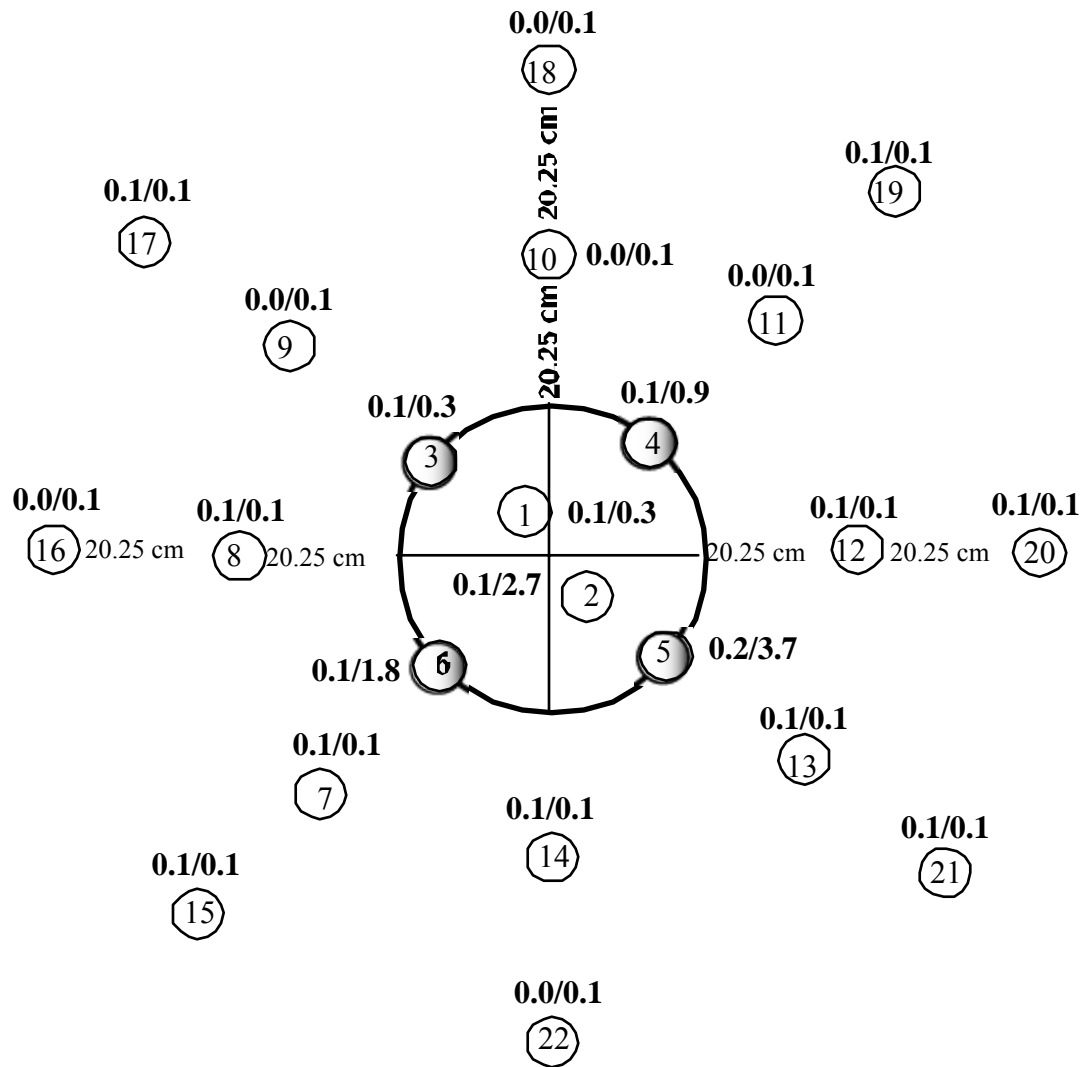


Figure 10. The location of 2.5 cm plugs in the Midlawn Plot A. The dead spot is indicated by the circle and measured 3.75 cm North to South and 3.5 cm West to East. North is at the top. The plugs radiated from the edge of the dead spot in 20.25 cm increments. The values are plant/soil ng of *O. herpotricha* DNA/g sample.

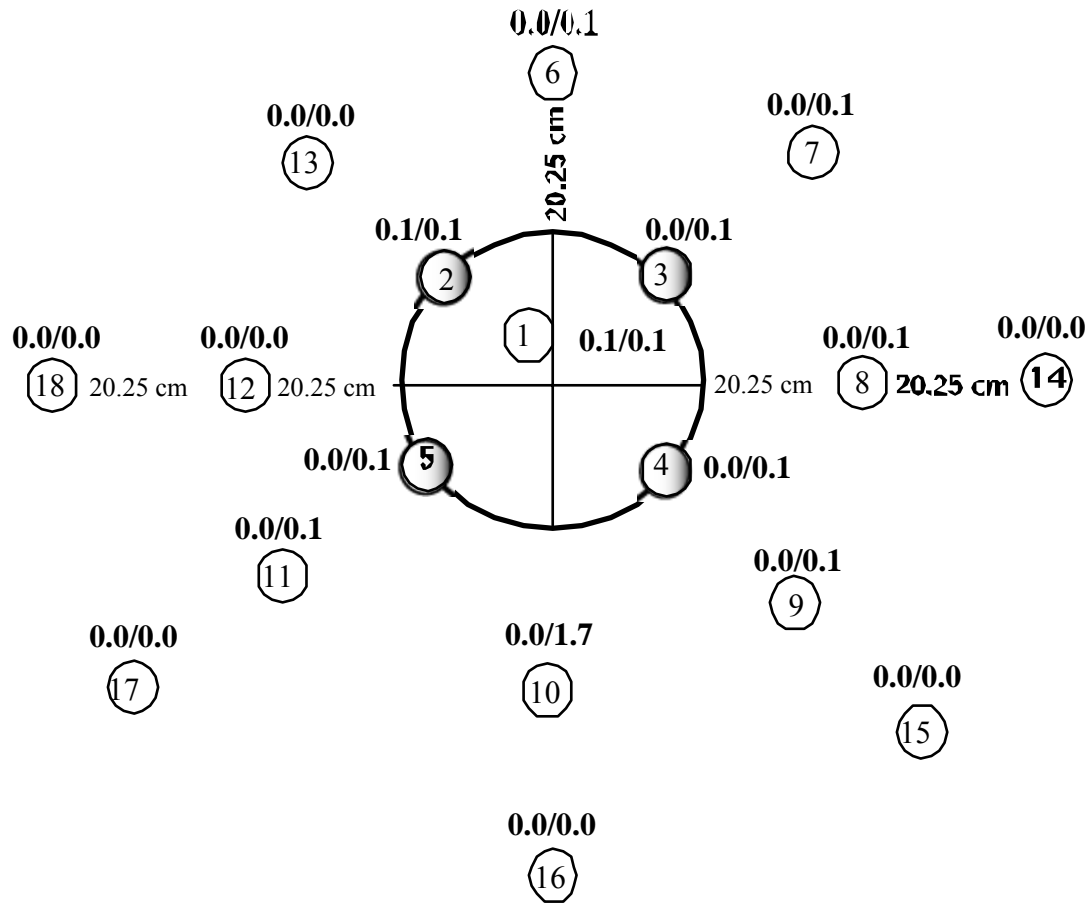


Figure 11. The location of 2.5 cm plugs in the Midlawn Plot B. The distribution of the plugs was influenced by the edge of the plot near the number six plug. The dead spot is indicated by the circle and measured 5.5 cm North to South and 3.25 cm West to East. North is at the top. Only one 1-inch plug was removed from the center of the small dead spot. The plugs radiated from the edge of the dead spot in 20.25 cm increments. The values are plant/soil ng of *O. herpotricha* DNA/g sample.

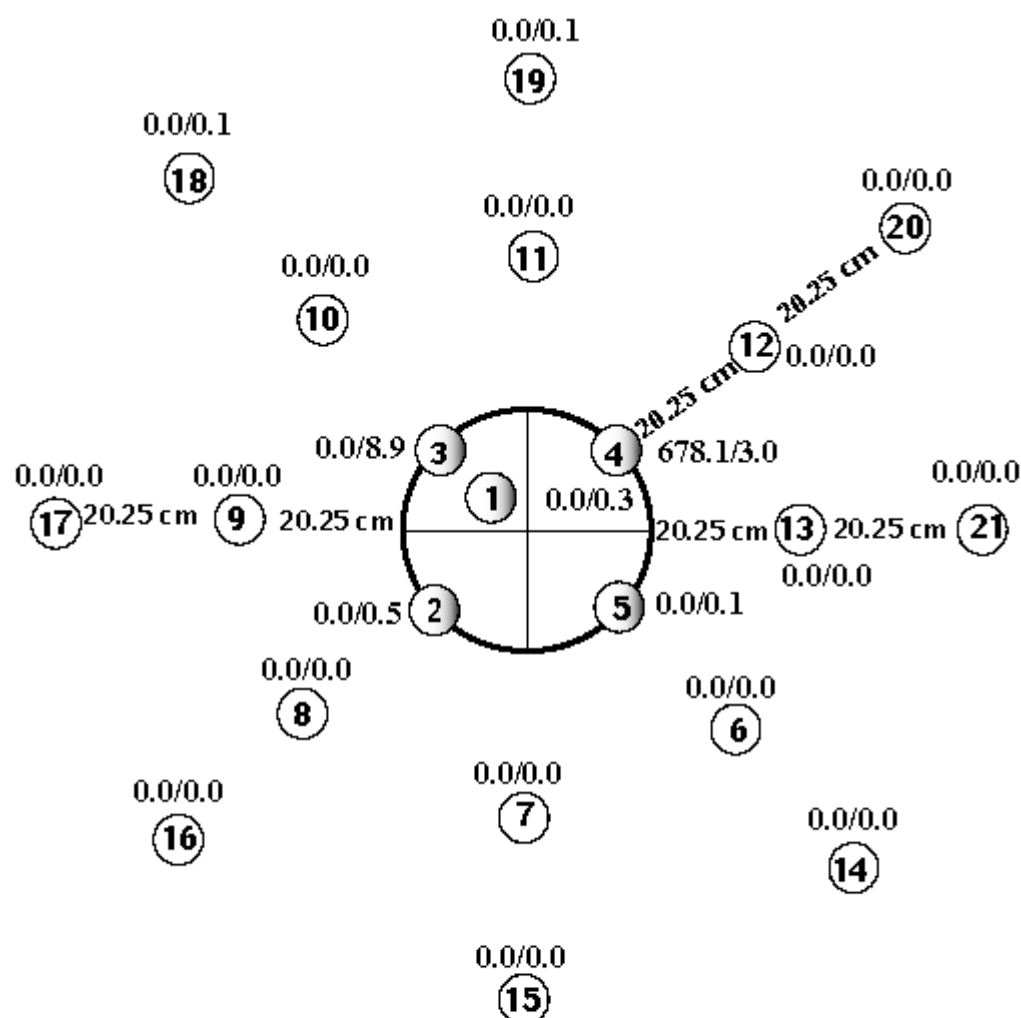


Figure 12. The location of 2.5 cm plugs in the Midlawn Plot C. The dead spot is indicated by the circle and measured 4.75 cm from North to South and 6.0 cm West to East. North is located at the top. Only one 1-inch plug was removed from the center of the small dead spot. The plugs radiated from the edge of the dead spot in 20.25 cm increments. The values are plant/soil ng of *O. herpotricha* DNA/g sample.

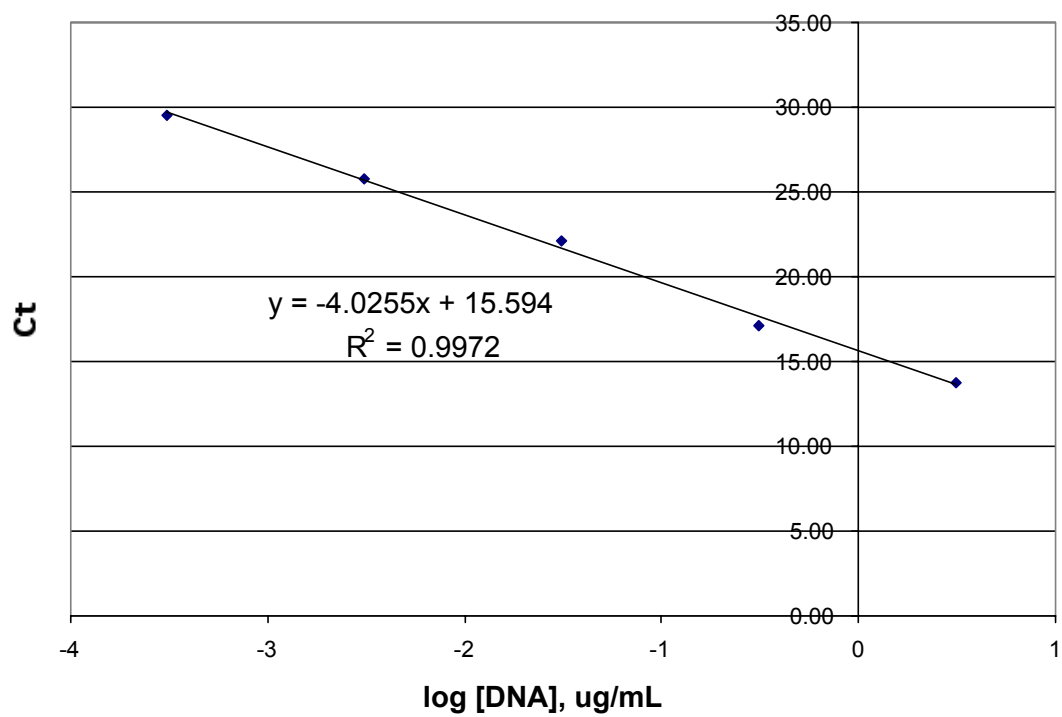


Figure 13. The standard curve of *O. herpotricha* genomic DNA, showing the relationship between DNA concentration and threshold cycle number (C_t).

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APPENDIX

Appendix 1. Soil parameters of the bermudagrass cultivar plots at the Oklahoma State University Turfgrass Research Center, Stillwater, Oklahoma. The soil was analyzed at the Soil, Water, and Forage Analytical Laboratory, Oklahoma Cooperative Extension Service, Oklahoma State University, Stillwater, OK.

Soil parameters:

Soil: Norge loam

Soil family: fine-silty, mixed, thermic udic paleustolls

Soil composition:

32.5 % sand

42.5 % silt

25.0 % clay

7.2 pH

Organic matter classification:

High levels, 3.1 % organic matter

Nutrients:

Surface nitrate 7.5 mg kg⁻¹

Surface sulfate 9.0 mg kg⁻¹

Magnesium 578.5 mg kg⁻¹

Biologically available potassium 62 mg kg⁻¹

Biologically available phosphate 300 mg kg⁻¹

Micronutrients:

Iron 53.3 ppm

Zinc 1.90 ppm

Boron 0.79 ppm

Appendix 2. The 16S rDNA contig sequences of the 225 culturable endophyte bacteria isolated from the crown tissue of Midlawn and Tifgreen cultivars of bermudagrass. The asterisks indicate where the sequences were truncated to produce high quality sequences for analyses.

Endophyte: 1

N***AGGGTGCAGCGTTTCCGGCATTGTTGGGCGTAAAGAGCTCGTNNCGGTTAGTCGNGTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCCTGCACTGGGTACGGGCAGACTAGAGTGCAGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAGGG
TGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGG
TTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGACCCGCACAAGCGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAA
GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTGG***NN
AATGTTGNCNN
NN
NN
NN

Endophyte: 2

CCTNGTCTGAGNGTTGNGTTGTTTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAGGC
TCAACCTCGGGCCTGCACTGGGTACGGGCAGACTAGAGTGCAGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAA
TGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAGGGTG
GGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGGTTT
CCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAA
CGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTGTGAGAATGT
TG***NCNCNN
TNN
NNNTNN
NN

Endophyte: 3

GCTCNGTCGTGAGAAGTTGNTCTCGNGTCGGGAAAGTNGGCTCGTGTGCGGAAANGTTGCGTCTGCTGTGAAATCCCGA
GGCTCAACCTCGGGCCTGCACTGGGTACGGGCAGACTAGAGNGCGGTNGGGGAGATTGGAATTCCTGGTGTANCGGTG
GAATGCGCAGATNTCAGGAGGAACACCGATGGNAGAAGGCNGNATCTCTGGNCCGTNACTGACGCTGANGAGCGAAA
GGGTGGNGAGCNAACAGGCTTANATACCCTGGTATTCCACCNCANTNANTNTNNGAACTATNTNNGGNGGACCNNTC
GNCCGGCACCGCNNNGACCGTTTCNTTCCCCNTTTNGTTTCCCNNNCCCTGNGNNCCNCNGTNCGNTNANGTNC
NNACCTNANNNNGAGANGGACCCNNGACCCNCGCNACCCGGANNCNNANTGCGNGNTTTTCNTTGATGAACACGCNN
AAAANCACTTNNCAAAGGCNTANTNTTATANCNNNAGCCCTTCTANNANACTGTTCANCTNTTATCGCANCANNNCNN
CNTANNTANGNNGCTNCNTNGGTNNCCNCNCTCANTNNCTTNNNANNTTCNNTNCCCCNTCCNAT***NNNNNNNTNN
CGCCTNGCCTNNNNACCAANNANGTTNNTAANTNAAANCNGCGNNGTCNNCNCGATATNCNCCGNCNGNATANCATN
NCNTCTNGCGNNNTCANCCACNTCNTNCNGCCCATATNANCNNNNNNNNANCCANTACCTCGCCNNNANTCTNNGNN
CTCCNCCATGTTNNTAANATNNNACNNTTANNANNCNACAGCCNCCNNNAANTTANNTCNGNNNNANNAATGNANCC
CCCNCCNNNNNTNTCNGCTNNANNTANNN

Endophyte: 4

GTAGGCNCGGTCGNCGTTTCGCTTNNCTGCNCGTAAAGGGCNCNAGGCGGTNATTTAAGTCAGNTGTGAAATCCCGG
GCTCAACCTGGGAACTGCATTNGATACTGGCTAGNCTTGAAGTNTGGTAGAGGNGNNTGGAATTCCTNGTGTAGCGGT
GAAATGCGTAGATATNCGGAGGAACACCAATGGCGAAGGCNACCCNCTGGGCCNTTACTGACGCTGATGTCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACNCCGTAAACGATGNNNACTAGTTGTTGGGACCCCTTNCNT
TNTNTNCGNNGCTTACGCCTTANNNGTCCCCCTGGNGAGTAGCCGCCGCAAGNNTGAAACTNNAANGAATTGNCGGG
GGCCCCCNCNACCNNGGANNATGTTGTTTAATTNTANGCNACNCCAAAACCTTNCNCCTTTTGNATGCCNNGCA
NCCTTNAGANATANNNGTNTNCCTTTNNGGACACTGACACACAGGNNNTGCATGGTTGTCCACATCTCGTGTGCGAGAN
ATGTTGGGTTAANCCCCNNAACGANNCACCCCTTGNNTTATTTCCNCCNACANGTGGGTGNTATCTTATGGCNCNN
GCCNTGACAA***NNAGGAGGAGGGGGGACNANNNTNATNATCCCTTGNCCCTGGGGGNTCGGCCNCNTCTCTC
NGGTNCNCCNCGNCGNNGNCCNNAANGGGAAGGTANNCCANATCCCNANGCNNTCNGNCCNNTNGNNGCTN
CCTCTNN

Endophyte: 5

TCNAACACGGTAGCCGTAAGCTTGGCNCCTGGGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCGG
GGCTCAACCTGGGAACTGCATTGTGACTGCATCGTGGAGTACGGCAGAGGGGATGGAATTCCTGCGTGTAGCAGTG
AAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCTTGGGCCCTGACTGACGCTCATGCACGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTC
AGTAACGGAAGAACGCGTGAAGTTGACCGCTTGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGG
ACCCGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATC
CTTTAGAGATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGGTGTCGATGGCTGTCTGTCAGCTCGTGTCTGTAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTGCCATTAGTTGCTACGAAAGGGCACTTAATGGGACTGCCGGTGAC
AAACCGGAAGGAAGGTGGGATGACGTCAAGCTTGGGAGTACGGCCCTTATAGGTGGGGCTACACGTCATACAATGAGGTG
TACAGANGGTTGCCAACCCGCGAGGGGGAGCTAATCCCATAAAGCCAGTCGTAGTCCGGATCCAGTCTGCAACTCNA
CTGCGTGA

Endophyte: 6

AGNCNAACAGGTAGCCGTAAGCTTNNN***TCCCGGGGAAAGGGCNCGTAGGCGGACTTTTAAGTCGGAGGTGAAAG
CCAGGGGCTCAACNTGGAATTGCCTTCGATACTGGGAGTCTTGAGTTCGGAAGAGGTTGGTGAACTGCGAGTGTAG
AGGTGAAATTCGTAGATATTCGCAAGAACACCGGTGGCGAAGGCNGGCCAACTGGTCCGAAACTGACGCTGAGGCGCG
AAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGGGAGCTT
GCTCTTCAGTGGCGCAGCTAACGCTTTAAGCATTCGCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTG
ACGGGGGCCCCGACAAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGCAGAACCCTTACCAGCCTTTTGACATGT
CCGGTTTGATCGGCAGAGATGCCTTTCTTCAGTTCGGCTGGCCGGAACACAGGTGCTGCATGGCTGTCGCCAGCCTCGT
GTCGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTCGCCCTAGTTGCCATCATTAGTTGGGAACCTCTAN
GGGGACTGCCGGTGATAA***NCCGCGAGGAANGTGGGGATGACCTCAAGTCCTNATGGCCCCCTTACAGGCTGGGCTAC
ACACCTTGCTACAATGCCGNGACAATGGGCAACCNAAGGGCNACCNTCAACNTNTCCCCAAAAAGCCCNNTNANT
TTANATTGCACTCTTGCAACTNGAGTGCNTNGAA

Endophyte: 7

GGAGGTGCAGCGTTATCCGGCTTTATTGGGTTTAAAGGTCCGTAGGCGGATCTGTAAGTCAGTGGTGAATCTCACAG
CTTAACTGTGAAACTGCCATTGATACTGCAGGTCTTGAGTAAGGTAGAAGTAGCTGGAATAAGTAGTGTAGCGGTGAA
ATGCATAGATATTACTTAGAACACCAATTGCGAAGGCAGGTACTATGTCTTAACTGACGCTGATGGACGAAAGCGTG
GGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGCTAACTCGTTTTTGGGTTTTTCGGATTACAG
ACTAAGCGAAAGTGATAAGTTAGCCACCTGGGGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGGGCC
GCACAAAGCGGTGATTATGTGGTTTAAATTCGATGATCAACGCGAGGAACCTTACCAAGGCTTAAATGGGAAATTGATCGG
TTTANAAATAGACCTTNCCTTCGGGCAATTTTCAAGGTGCTGCATGGTTGTCGTCAGCTCGTGTGGGAAATA***NNT
TGGNNCCNNCACNNNNCNCNNNNCNCNNNNNGNNGNNNNANNNNNNGNNNNCNCNNNNNNANNNNNCNCNNNGNANNTG
NNNNGNCNN
ANNCCNNNGCNCNNNNCNCNNNTNCCNNCNCNN
NTNGCNTNNGNCNCNCNGNNNNNNNNNTANNNNNCCGNGNNC

Endophyte: 8

GGANTTCAGCGTTNATCGGATTANTGGGCGTAAAGCGCACGCAGGCGGTCTGTAAAGTCAGATGTGAAATCCCCGGC
TTAACTGGGAAGTGCATTTGAAACTGGCAGGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCANGTGCNAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCACTTGGAGGTTGTTCCCTTGAGGAGTG
GCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAAATTCGATGCAACGCGCAAGAACCTTACCTACTCTTGACATCCANAGA
TANCAGAGATGCTTTGGTGCCTTCNGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCANCTCGTGTGCGAAAAAGT
TGGC***NNCNCNCNCNNNGCCNNGGANATNNANANNNGANNNNANNNAAAGCCNNGNGNTANNNGTGNNTCNGAGT
GCNAAGNGNANCCGCNNNGGNTNCNCGNANGCCNGNAANGTNTATNTCNGATTGCGCNCNACANATNTCNCNCN
GNGCAGTNNNNGACNCNCGCNCACANCCNCCANNCANANANTCNGCCNCCNCTNCACNNCNCGTNCNNNCAGN
NCGTNCNGGCCNACNCNNCNCNNGNCCGNNNCAN

Endophyte: 9

TAGGGTGAAGCGTTAATGCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCG
GGCTCAACCTGGGAAGTGCATTTGTGACTGCATCGTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTG
AAATGCGTAGATATGCGGAGGAACACCNGATGGCNGAAGGCAATCCCTGGGCGTGTACTGACGCTATGCACGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGAC
TCAGTAACGAAGCTAACGCGTTAAGTTCGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGG
GACCCGACAAGCGGTGGATGATGTGGTTTAAATTCGATGCAACGCGAAGAACCTTACCCACCTTTGACATGTACGGAAT
CCTTTAGAGATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCG***NGN
AAAANTTTNGGNN
NN
NN
NN

Endophyte: 10

GGAGGGNGCNGCNGNNANTACGCNTTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTAAAGTCAGATGTGAAATCCCC
GGGCTTAACTGGGAAGTGCATTTGAAACTGGCAGGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCAGGTGCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCACTTGGAGGTTGTTCCCTTGAGG
AGTGGGTTCCGGAGCTAACGCGTTAAGTCGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAG
AACTTAGCAGAGATGCTTTGGTGCCTTCGGGAAGTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGTGAAA
TGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTATCCTTTGTTGCCAGCGATTCCGGTCGGGAAGTCAAAGGAGACTGC
CGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAA
TGGCGCATACAAAGAGAAGCGACCTCGCAGAGCAAGCGGACCTCACAAAGTGCGTCTAGTCCGGATCGGAGTCTGC
AACTCGACTCCGTGAAGTC

Endophyte: 11

GNTNNTGCAGCGGGTANTNGGCATTACTGGGCGTAAAGCGTTNNCCAGGCGGGTGATAGTAAGTACAGATGTGAAATC
CCCGGGCTCAACCTGGGAACCTGCATTTGTGACTGCATCGCTGGAGTACNGGCAGAGGGGGATGGAATTCGCGGTGTA
GCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCTGGGCGTGTACTGACGCTCATGCAC
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTT
CACCTGACTCAGTAACGAAGCTAACGCCGTGAAGTTGACCGCTGGGGAGTACCGGCCGCAAGGTTGAAACTCAAAGG
AATTGACGGGNCCCGCACAAAGCGGTGGATGATGTNGTTTAAATTCGATGC***AAAANGAAAAACCTTNTNACCTTTG
NNATNNNCTTGAAAAACTTTAGGGGGGNGNNGAGAGNCCNAAAAAACCCTTCAATCTTTTGG
GGGAAATTTNNNNNTTTTNTNNNAAACNTNTTTTNCNNNGNTTNGNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NN
NN
NN

Endophyte: 12

TAGGGCGCAGCGTTTCCGGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTGTGCGCTCTGCTGTGAAATCCCAGGGCT
CTCAACCTGGGCACTGCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCCTGGTGTAGCGGTGGAAT
GCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCGTAACTGACGCTGAGGAGCGAAAGGGTGG
GGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGGTTTC
CGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGA
CCCCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAAC
GGGCCAGAAATGGTCAACTCTTTGGACACTCGTAACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCGGAAAAATNTT
TGCC***NN
NTNNNTTTNNNNNNANNNNTAANNNTCNTGNANNNNNANANAGNGANNNGNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNTATNTTNNANNTNGCNTNATANNNNNNNANNANCCNNNCNNNTTNNNNNNNNNTNNANNNNTNTNTANNNCNNTT
TANNTTTNNNGNCN

Endophyte: 13

TAGGGTGCAGCGTTAATCGGATTACTGGGCGTAAAGCGTGGCGAGGCGGTGATGTAAGACAGATGTGAAATCCCCGGG
CTCAACCTGGGAACCTGCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGAA
ATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCTGGGCGTGTACTGACGCTCATGCACGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAG
TAACGAAGCTAACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGAC
CCGCACAAGCGGTGGATGATGTGGTTTAAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATCCT
TTANAGATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTGGAATAATG
TTNG***NN
NN
NN
NN

Endophyte: 14

TACNNCACGGTNGNTCGTAAGGCTTGNTCNCNNGGTAAAGCGTGGCGAGGCGGTGATGTAAGACAGATGTGAAATCCC
CGGGCTCAACCTGGGAACCTGCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTA
TGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCTGGGCGTGTACTGACGCTCATGCACGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGAC
TCAGTAACGAAGCTAACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGG
GACCCGACAAGCGGTGGATGATGTGGTTTAAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAAT
CCTTTAGAGATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTGCGTGA
TGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTGCCATTANTTGCTACGAAAGGGCACTCTAATGGGACTGGCGGTGA
CAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTACATAAATGGCTG
GTACAGAGGGTTGCCACCCCGCAGGGGGAGCCTAATCCATAAAGCCAGTCTGATGTCGGGATCCGCAG***NCNTG

Endophyte: 15

CTCTGTCGTGAGANGTTGAGGATTACTGGGCGTAAAGCGTGGCGAGGCGGTGATGTAAGACAGATGTGAAATCCCCGG
GCTCAACCTGGGAACCTGCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGA
AATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCTGGGCGTGTACTGACGCTCATGCACGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCA
GTAACGAAGCTAACGCGTGAAGTTGACCGTCTGGGGAGTACNGCCNAGGTTGAAACTCAAAGGAATNGACGGGGA
CCCGCACAANCGGTGGATGATGTGGTTTAAATTCNATGCANCGNAAAAACCTTACCCNCCTNTNACATGNACGGAATN
CTTNTAGATANACNATTTGTTCCAAAGAATAACCTGTANACNANGTGTGCATGGNCTNCTNCNANNCT***NN
TNCCNNANTTTNCNTNNTCNNNCNNNCNANNNNACCCNANTCTTNNNACNTCTANCCTNATCNNNCATNCNNATGN
NNTTNCATCCNANTNTCANTTCTTTCNNNCNNTTTCNCCANCCNCCNCCGNNCNGNCTATCANTCTNNCANCCN
CANNAANNNCTGCACTNTNCCNATTNNNTNNNTTTTATNTCTCNCNCTCTNTTCTCNTACTNCAACANANNNGNC
NGNAACNCCGNTCCNANANNATCNNNCNANCNCNGNTACCNNTCGNNTATT

Endophyte: 16

CTCTGTCTGTGAGCNGTTGACTNNNTTCTGGAAATTNGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAACCTGCATTGAAAACCTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACAAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCACTTGGAGGTTGTGCCCTTGAGGCGT
GGCCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAA
CTTNNCAGAGATGNNTTGGTGCCTTCGGGAACCTCTGANACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCG***NNCN
AANNNTTGANTTTTTCTCCNNNNNCNTCAATNNNNNCNCCNCCNCTTTCTNNTTTTNATANCTNCNNTNNCNNTNCN
CCNTTCNNCCNNTTTNTCNTNTTCCCCNCTNNNTNANCCCCNCCCATCTTCTNNCTTTCACCCCCCTCNCCTANNAT
CNNNNNTTACTNANATNNCTGCANNNTNCNNNTNTATTATNTNTTATCTCTTCNANTNNNNNCNATNNNACCNCANNC
NTANTNGTNTNNCTACNNNTNNNNCNGNCTNNTANATCNN

Endophyte: 17

CTCNGTCTGTAGNTGTTGCGNATTATTGGGCGTAAAGGAGCTCGTATGCGGNTNGTCGCGTCTGCTGTGAAATCCCGAG
CTCAACCTGGGCGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGG
TGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCACGG
TTTCCGTGACGCAGCTAACGCATTAAGTTCCTCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGA
GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTC***NCTCGTGTCTGTGG
AAATGTTGCCNNNNNNNTTNCNCTCNCNCCNCTTNNNTNNCCNNTTCCNTTANCTANTNTNNNATNACNNNTATCNN
NNTTNCCTTNTTNCNACCNNCNCNNNCANNNNNNTNNCNCNNTNTTNTNTCNTCTTNCNCCNCTNATNNNNNC
NNTNNNNCNCNCCNCTNTNNNNNANNNNANNCNNNNNTCTNNANANNNCNCNCCCNNTNTNTNNNNCCNNTCNN
GCCCCNATNNCNC

Endophyte: 18

TAGGGTGCAGCGTTAATCGGATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCGGG
CTCAACCTGGGAACCTGCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGTGTAGCAGTGAA
ATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCTGTACTGACGCTCATGCACGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAG
TAACGAAGCTAACGCGTGAAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGAC
CCGCACAAGCGGTGGATGATGTGGTTAATTTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATCCT
TTAGAGATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGNAAAAAT
GTTG***NN
NN
NN
NN

Endophyte: 19

TAGGGTGCAGCGTTAATCGGATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCGG
GCTCAACCTGGGAACCTGCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGTGTAGCAGTGAA
AATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCTGTACTGACGCTCATGCACGAAAGCGTG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTC
AGTAACGAAGCTAACGCGTGAAAGTTNACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGTGGATGATGTGGTTAATTTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATC
CTTAGAGATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGNAAAA
AGTTGG***NN
NN
NN
NN

Endophyte: 20

GGAGGTGCAGCGTTATCGGATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
TCAACCTGGGAACCTGCATTCGAAAACCTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACAAAAGACTGACGCTCAGGTGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCACTTGGAGGTTGTGCCCTTGAGGCGTG
GCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGG
CCCGACAAGCGGTGGAGCATGTGGTTAATTTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTT
NCCAGAGATGNNTTGGTGCCTTCGGGAACCTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGGNAAAAATGTT
GG***NN
NN
NN
NN

Endophyte: 21

CGCTCTGTCGTGAANNNGTTGANNATTATNNGGNGNANNNGAGCTCGTGNCGCGGNTTGTNGCGTCTGCTGTGAAATC
CCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGGTAGGGGAGATTGGAATTCCTGGTGTAGC
GGTGGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGA
AAGGGTGGGGAGCAAACAGGCTTANATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTC
CACGGTTTCCGNGACGCAGCTAACGCATTAAAGTTCCCNCTGGGGAGTACGGNCGCAANGCTAAACTCAAAANGAA
TTGACGGGGACCCGCACAAGCGGCGGAGCATGCGGGATTAATTNATNACNCCNCGAANAACCTNACCAANGCTTGAC
ATATACNNACAACGGGGCCAGAAATGGTCAANTTTTGNATACTCCCTNAAACNGGCNGGCNCTGNTTGTNTTCNNCT
NCTNTACNCGAGAATGTNTNTTCTTCNNTT***NNNNCNTCNCCNCANTTNTNCCCNCCNCCNNNNCNCTCNTNGTTT
TTNNNTNCCTANNNTNTCCGCCANNATCCCGATTNGTAGNTNTNNCNCNTAGACTNNTANANTTCNNNATNTNNNN
NCTANNTNNNNCTCNTNCTCTNNTNTANNANTNNNTNNNATNCCCCNGATCNTCATTNNAATTATNNNNNACTNA
NNCNNTANNCTNCCNCTNTACAATTANNNTNCCCCNCCNNTNTAANCNNNNAAATTTTANNT

Endophyte: 22

GGAGGTGCAGCGTTATCGGATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGC
TCAACCTGGGAAGCTGCATTGAAACTGGCAGGCTAGAGTCTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCNGGCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCGACTTGGAGGTTGTGCCCTTGAGGCGT
GGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCCTACCTACTCTTGACATCCAGAGAACT
TNCCAGAGATGNNTTGGTGCTTCGGGAACCTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGGNAAAATNT
TGG***NN
NN
NN
NN

Endophyte: 23

CGAGCTCGTGTGAGCAGTTGTTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGGG
TGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAAGTAGTTGTGGGGACCATTCACGG
TTCCCGTGACGCAGCTAACGCATTAAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACG
GGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCCTACCAAGGCTTGACATACGA
GAACGGGGCAGGAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCGNAAN
TGTG***NN
NN
NN
NN

Endophyte: 24

GGAGGTGCAGCGTTAATCGGATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCAGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCGACTTGGAGGTTGTGCCCTTGAGGCGT
GGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCCTACCTACTCTTGACATCCAGAGAACT
TNCCAGAGATGNNTTGGTGCTTCGGGAACCTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGGNAAANATNT
GG***NN
NN
NN
NN

Endophyte: 25

CGAGNTCTGTCGTGANAGTTGN***TTTGGCGTNAAGAGCTCGTACGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAGG
CTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
ATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGANNNGGT
GGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAAGTAGTTGTGGGGACCATTCACCGT
TTCCGTGACGCAGCTAACGCATTAAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGG
GGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCCTACCAAGGCTTGACATATACGAG
AACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCTGAGAAT
GTTG***NCCNN
NNNNNTNNNNNNANNTTNNNTNTNNNNCNCTCTNNNTNCCNNNNNNNNNTNTNNNNNNCCNNCNAANNTNNNN
NNNNNNNTNNNCNN
NNNNNTCNNNNNN

Endophyte: 26

CTCNGTCTGAGTGTGANN***CATTTNNGGCGTAAAGGAGCTCGTANGCGGTTTGTGCGCTGCTGCTGTGAAATCCCGA
GGCTCAACCTCGGGTCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTG
GAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAGG
GTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACG
GATTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGAC
GGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACG
AGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATNTTGTCTGTCAGCTCGTGTCTGT***NA
AANTGTTGNCNNNNCNCNTNNTANNACANNN
NNNGNTNCNNNNNTTNTNNTCNGCNN
NNTCNCNN
NCNACNNCNAATNNNNNTNTN

Endophyte: 27

GCTCGGTTCTGTTGNNATTTTGGATTTCNGGTNCNGNAGTNTTNGCCNCGGTTCCGGNAATNTTGGCCCCGNNCCGGNN
NTTGGCCCCCTCCCGNNNNNTTTCCTCNCNCGNNATTTTNTCCNANCNNCGGGNANTNGTNTTTCNNTTGTN
GGAGNNGANTNCTTCTNTATNACNGNGGNNNNCCGGNTGGGGGNAANGNTGTNNNATNGGNGCTGGGGACGTGCC
CNTTTNCCNCCTATATNGNTTGATNTGAGNGGGGGGGGCTGGTNCATCNCNGGNNCGTTTGTGGNCNTNNTNNGC
CNCNTNNAANNTTAAACNNNGGGANGNCCNGNGACCTTNTCTTTTNNCNCNNNNNNNNNNNNNNNNNNNNNN
TNNCNCNTGGANNATTAGTCNNNNNATTCNTANTCAGTTTATGNNGGNTTACNCNANGCNCNNNTNNCNCNNCNGTANG
NNNGCGNCNGNGNTCNCATNNNNNNCNCANTGTNCCGGTANACNTANCNTNNGNTNNNAGNGTTGTTGCGNGTN
TTCNTNTCGCGNNCNGTNNNNATTNCATCTNCNANNTTNTANGCNCNNGGGTCNGCGNCNCNTNCCNTGGNCTCCAN
NANCANGCNCNNNNANCCCGNNAATNNCNCNTGTTTNTNNNNNGNNNNNNNGCCCATNGNCCNNNNNGCCTCCNCNN
GNNGGCGNCNNATTTTNTNNGCGTTGNCNCNNCTCCCCGCTNNNNCNCGATATTTNCTNNNGCNCNCNTTTNNTCTGN
NTNNNTNTNNTNTATATGTCNNNANTCCNNCCNTGCNNGTGTGTCNTATNNCCNTCTCCTTCGTANNNGCG
CGTANGNNNNNTTANATATGTTNCNNCTCCGNCNGTGNNTGCGCCNCCNNCNCNNCCACCNNNNNNNTATNTTCGCC
CACNTTNTNACANTNANTNATATNT

Endophyte: 28

CTCGGTCCNGAGNNATGCGGN***ATTTTGGGCGTAAAGAGCTCGTANGCGGNTNGTCTGCGCTGCTGCTGTGAAATCCCGA
GGCTCAACCTCGGGTCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTG
GAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAGG
GTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACG
GATTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGAC
GGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATNTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAC
GAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGTTGTCTGTCAGCTCGTGTCTGT**
*NAANTGTTGGNCNN
NNNTCNCNNNTTNTNNTTNN
NCCNNTNN
NNNNNNNCCNCCNCNCTNCCACNANNTNNNN

Endophyte: 29

CTCNGTCCNGAGNGTTGCGGCATTATTGGGCGTAAAGAGCTCGTGTGCGGANATNTTGGTCTGCTGTGAAATNCCGAG
GCTCAACCTCGGGTCTGCAGTGGGTACGGGCAANACTAAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCTGCAATATATCAGGAGGAACACCGGATGGCAAAGGCAGATNTTGGGCCGTTACTGACGCTGAGGAGCGCA
AAGGTGGGGAGCAAACAGGCTTAAATACCCTGGTAGTCCACCCGTAACNTTGGGAACCTANTTGTGGGGTCCATT
CACGGATTCCGTGACGCAGCCTAACGCATTAAGTTCCCGCTGGGGAGTACNGCCGCAAGGCTAAAACTCAAAGGAA
TTGACGGGGACCCGCACAAGCGGCGGANCATGCGGATTAATTCGATGCAACGCGAANAACCTTACCAAGGCTTGACAT
ATACNAGAACGGGCCAAAAATGGTCAACTCTTTGGACACTNTAAACAGGTGGNGCATGGTTGTCTGTCANCTCGTGT
GTGAAAAATGTTGAC***NNNNNNNNCNCNN
NNNNNNNNNNNNCNCNNNNNTNNNNNTNNNNNNNNNNNTTNNCNCNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNATNN
NNNNNNNNNNNNCNCNNNNNT

Endophyte: 30

GAGGTTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCG
GGCTCAACCTGGGAAGTGCATTCAAAACTGTGAGCTAGAGTATGGTAGAGGGTGGTGAATTCCTGTGTAGCGGTG
AAATGCGTAGATATAGGAAGGAACACCAAGTGGCGAAGGCGACCACTGGACTGATACTGACACTGAGGTGCGGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACCGCTAAACGATGTCAACTAGCCTGTGGGAGCCTTGAGCT
CTTAGTGGGCGAGCTAACGCATTAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGG
GGGCCCCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGA
ACTTTCAGAGATGGATTGGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCTGTCAGCTCGTGTGCGNAGAAT
GTTTGTAT***NTNNACCATNTNTNTATNNCTCNCCTNNNNATNTNCNNCNCNTNNNANATACNCCCTNATNCC
CTTNTCTCNCATNTCTTNCNCTNTNTCTNTCTNTCTNTCTNTCTNTCTNTCTNTCTNTCTNTCTNTCTNTCTNTCTNT
NCCTTTTCTAGNNTANTATNTATCGNNANTCNCNNNNCANCNCCCNCTTACNTACCTCNCNTCCNTANCTNCCC
NCCTN

Endophyte: 31

N***GCTCTNTCGTGAAANNNTTGANCCNTTTNNNGAGNANTGGCTCNGTANGCGGATTGTTNCNTCTGCTGCGAAATNC
CNAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGGTAGGGGAGATTGGAATTCCTGGTGTAGCG
GTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGAGCTGAGGAGCGAA
AGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCC
ACGGTTTCCGTGACGAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATT
GACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAANAACCTTACCAAGGCTTGACATAT
ACGACAACGGGCGAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGNAT***NNNCNAANNCTNNNNACCACANTTCCCCCNCNCNCANATNNTNCTTTTTCTTTNTNCNCNTCC
NCCAGTNTNCCTANNTNTCNATTTCTANCNNANANCNCACTNTNCTCNCNCTNTCCNCTNTNTCCGNNNTGCCNCAN
ACCTNTCNTGTNANCNATNNNCCNCCANANCCNCTNTNANTTTCTTNTNCNNNTTNTCTANANTCATTCTNTTANN
NTCCCCCTCNACNCCCCCTTATNCTCGN

Endophyte: 32

AAAGGTTNCTCGTAAGCTTTGGATCCCCGGGGCGCGCTAGGTGGTTCGTAAAGTTGGATGTGAAAGCCCCGGGCTCAAC
TAGGGTCAACCTGATTCAAAACCTGTCGAGCTAGAGTATGGTAGAGGGTGGTGAATTCCTGTGTAGCGGTGAAATGCGT
AGATATAAGGAAGGAACACCAAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAG
CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGC
GCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGGCCGCA
CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGCCTTGACATCCAATGAACCTTCCAG
AGATGGATTGGTGGCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGAGATGTTGGGTTA
AGTCCCGTAACGAGCGCAACCCTTGTCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAA
ACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGGTACACACGTGCTACAATGGTTCGGTA
CAGAAGGTTTGCCAANCCGCGANGTGGAGCTAATCCCACAAAACCGATCGTATCCCGGATCGCAATCTGCAACTCGAC
TG

Endophyte: 33

TAGGGTCGCGTNGTTTCNTCGGCNTNACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTAAAGTTGGATGTGAAAGCCCC
GGGCTCAACCTGGGAACCTGCAATTCAAAACCTGTCGAGCTAGAGTATGGTAGAGGGTGGTGAATTCCTGTGTAGCGGT
GAAATGCGTAGATATAGGAAGGAACACCAAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGC
TCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACG
GGGCCCCGACAAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGCCTTGACATCCAATG
AACTTCCAGAGATGGATTGGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGAGAG
TGTTGGGTAAAGTCCCGTAACGAGCGCAACCCTTGTCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTG
CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGGTACACACGTGCTACA
ATGGTTCGGTACAGANGTTGCCAAGCCCCGANGTGGAGCTAATCCCACAAAACCGATCGTAGTCCGGATCGCAGTCTG
CAC

Endophyte: 34

GAGGGTGCAAGCGTTAATCGGATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTAAAGTTGGATGTGAAAGCCCCGG
GCTCAACCTGGGAACCTGCAATTCAAAACCTGTCGAGCTAGAGTATGGTAGAGGGTGGTGAATTCCTGTGTAGCGGTGA
AATGCGTAGATATAGGAAGGAACACCAAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTC
TTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGG
GGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGCCTTGACATCCAATGAA
CTTCCAGAGATGGATTGGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGNAAAAAA
NTTTTG***NN
NN
NN
NN

Endophyte: 35

GGTGCAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTAAAGTTGGATGTGAAAGCCCCGGGCT
CAACCTGGGAACCTGCAATTCAAAACCTGTCGAGCTAGAGTATGGTAGAGGGTGGTGAATTCCTGTGTAGCGGTGAAAT
GCGTAGATATAGGAAGGAACACCAAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTA
GTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCG
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGCCTTGACATCCAATGAACCT
TCCAGAGATGGATTGGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGAGATGTTG
GGTTAAGTCCCGTAACGAGCGCAACCCTTGTCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGGTACACACGTGCTACAATGGT
CGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCACAAAACCGATCGTAGTCCNGATCGCAGTCTGCAACT
CGACTG

Endophyte: 36

GGTGACGCGTTATCCGGATTTATTGGGTTTAAAGGGTCCGTAGGCGGATCTGTAAGTCAGTGGTGAAATCTCACAGCTT
AACTGTGAAACTGCCATTGATACTGCAGGTCTTGAGTAAGGTAGAAGTAGCTGGAATAAGTAGTGTAGCGGTGAAATG
CATAGATATTACTTAGAACACCAATTGCGAAGGCGAGGTACCTATGTCTTAACCTGACGCTGATGGACGAAAGCGTG
GAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGCTAACTCGTTTTTGGGTTTTTCGGATTACAGAGAC
TAAGCGAAAGTGATAAGTTAGCCACCTGGGGAGTACCGTTGCGAAGAATGAAACTCAAAGGAATTGACGGGGGCCCGC
ACAAGCGGTGGATTATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGGGAATTGATCGGCTTA
GAAATAGACCTTCCTTCGGGCAANTTTCAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCG***NGAAAAATNTTTGCNCC
CCCNCCNTNTGCCNCGGACCNCNATNNCCCCGNNGNTCNCCNGNNTNTTNCNCCCCCNCCNNTTNCNCCNT
CNNTATNTAATNNNTCNNTNTNGNCTTTCGTNNTTNNCNCNCCGANANCTNCTAACANCNCCNNTNCNCCNTNNNTNTNC
TNNNANTNNNTTNCNCCGNNTTNTNTNTTCCNNTNATTCTNTAACNCTAATNCNTNNCCCCNTNC

Endophyte: 37

CTCNGTCGTGAGAGTTGN***GGATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATTGAAAACCTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTG
AAATGCGTAGAGATCTGGAGGAATACNCGGTGGCGAAGGCGGCCCTGGACAAAGACTGACACTGAGGCACGAAAG
CGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCACTTGGAGGTTGTGCCCTTGAGG
CGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGAGTACGGCCGCAAGGTTAAAACCTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAG
AACTTNCAGAGATGNNTTGGTGCCTTCGGGAACCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCG***N
AAATGTTTGNNN
NN
NN
NN

Endophyte: 38

NN***TGGTGNCNGATAGTTGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAAGTCTGTTGTGAAAGCCCTG
GGCTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGAATTCCTCGGTGTAGCAGTG
TAAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGCGAGTACCTGGACCAACACTGACACTGAGGCACGAAAG
GTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGC
ACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATGTCGAG
AACTTTCAGAGATGGATTGGTGCCTTCGGGAACCTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCG***NAG
AANTTGTGANN
NN
NN
NNNNNNNNNNNNNTNNNNNNNNNNNNNNNTNTNNNNNNNNNN

Endophyte: 39

CTNTGGTGNN***GNGANAGTGTGNATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAAGTCTGTTGTGAAAGCCC
TGGGCTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGAATTCCTCGGTGTAGCAG
TGAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGCGAGTACCTGGACCAACACTGACACTGAGGCACGAAAG
GCGTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTG
GCACGCACTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATGTCG
AGAATTTCAGAGATGGATTGGTGCCTTCGGGAACCTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCG***N
*NAAAAANGTTGNN
NN
NN
NN

Endophyte: 40

AAGGTGCAAGCGTTCTCGGATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAAGTCTGTTGTGAAAGCCCTGGGCT
CAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGAATTCCTCGGTGTAGCAGTGAAT
GCGTAGAGATCGGGAGGAACATCCATGGCGAAGCGAGTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTG
GGAGCAAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGC
AGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATGTCGAGAACTT
TCCAGAGATGGATTGGTGCCTTCGGGAACCTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGANAATAATTT
NGG***NN
NN
NN
NN

[illegible][illegible][illegible][illegible][illegible]

Endophyte: 46

GCTCGGTGTCNGNNGNNTTGCNATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTTGTGAAAGCCCTGGG
CTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGAATTCCCGGTGTAGCAGTGAA
ATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCAC
GCAGTATCGAAGCTAACGCGTTAAGTTCCGCCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATGTCGAGAA
CTTTCAGAGATGGATTGGTGCCTTCGGGAACCTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGAAAAATG
TTGG***NNNNNGNNNNNNNNNNNNNNNTTCCCGNNGNNTNNNNNNNNNNNNNNNGNNNNNNNNNNNNNNNNC
NNNNNNNTNNNNCNNTCNNNNNNNNNNNGCNNNNNNNNNNNNNTNNNNNGNTTNGNNNNNNNNNNC
NNNNNNNNNNNNNNNNNNNNNGGNGNNNNNNNGNNNNNANNGNGGNNNNNGNNNNNNC
NNNNNTANNANNCNCGN
GNNNNCCNCCNTNNNNNTN

Endophyte: 47

CGCTCNGTCGTGAGGNGTTGANNATTATNNGGNGTANNGAGCTCGTAGGCGGNTTGTGCGCTGCTGCTGTGAAATCCC
GAGGCTCAACCTCGGGCTGCAGTGGGTACGGCGAGACTAGAGTGGGTAGAGGGTAGTGAATTCCTGGTGTAGCGG
TGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAA
GGGTGGGGAGCAAACAGGCTTANATACCCTGGTAGNCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCA
CGGTTTCCGTGACGCANCTAACGCATTAAGTTCCCGCCTGGGGAGTACTGCCGCAAGGCTAAAACTCAAAGGAATTG
ACNGGGGACCCNCACAANACGGGTGGAGCATGCCGAATTAATTTGATGCANCCNAANAACCTTACCAANGCTTGACA
TATTCNAAAAACGNGGCCCAAAAAATNGTNCAACTNTTCTGGANCCCTCGTAAACNNNGGTGGGTGCAANGGCTNTTT
CNCACCTCNTGTCTTNAAGAANTTTNANCCNNCTCNTTCTATNANTCANNTNTNNNNNANTANTNTATTANCNN
TNCATNCNNATGTAACCGATNNCNCNTTTTCTTNTNTCTCTNCCTANTNTCCCTCTATNTNCNNNNCTNTTCCCC
CNATNTAGNCNTTCTNTANTTCCNNNGATTNTNTNNNAATTTNANTNNCNAANNCCCCCCCCCTNTANCCNNNCNA
TNTTTNTCTNCCNCNCNNAANNCCCTTTNTTCCNNT

Endophyte: 48

GTCTACAAGGTAGCCGTAAGCTTGGCNCCTGGGTAAAGCGCGCGTAGGTGGTTTCGTTAAGTTGGATGTGAAAGCCCCG
GGCTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTATGGTAGAGGGTAGTGAATTCCTGTGTAGCGGTG
AAATGCGTAGATATAGGAAGGAACACCAAGTGGCGAAGGCGACCACTGGACTGATACTGACACTGAGGTGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCT
CTTAGTTGGCGCAGCTAACGCATTAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGG
GGGCCCCACAAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCCTTGACATCCAATGA
ACTTTCAGAGATGGATTGGTGCCTTCGGGAGCATTTGAGACAGGTGCTGCATGGCTGTCGTGAGTCTCGTGTGAGAT
GTTGGGTAAAGTCCCGTAACGAGCGCAACCTTGTCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGC
CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACAGCTGCTACAA
TGGTCCGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCAAAAACCGATCGTAGTCCCGATCGCAGTCTGC
AACTCGACTGCGTGAAG

Endophyte: 49

AAGGTGCAAGCGTTCTCGGATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTTGTGAAAGCCCTGGGCT
CAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGAATTCCTGGTGTAGCAGTGAAAT
GCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGC
AGTATCGAAGCTAACGCGTTAAGTTCCGCCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGG
CCCCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATGTGAGAACTT
TCCAGAGATGGATTGGTGCCTTCGGGAACCTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGAAAAAATT
TTGG***NN
NN
NN
NN

Endophyte: 50

GCTCGTTTGTGAGAGTTGANNATTACTNTGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTTGTGAAAGCCCTGGG
CTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGAATTCCTGGTGTAGCAGTGAA
ATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAANCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCAC
GCAGTATCGAAGCTAACGCGTTAAGTTCCGCCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATGTGAGAACT
CTTTCAGAGATGGATTGGTGCCTTCGGGAACCTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGT***NNA
ATGTTGNCNCCNCTCNCNCGNCACCTTNNNCNNNCNCTCCNATNTCTNNNNNTNNNGNNNCTCNCGTTTCCCNNTN
CCCNNTTTTNCNCGCTCGNCTANNGTTCGANCTNNNCNCCNCGNCCNNNNNTNTCCNNNNNGNCCNENATATCCNG
NNANNCNNTCNCNCCNNNNATNTNNNTTNCAGCTTCNTGNNGTCTTNNNNACGCCCTNNANTTTNTTCTCNCNCCN
CNCNCCATCNCNTNNAATN

Endophyte: 51

GGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAAGTCTGTTGTGAAAGCCCTGGGC
TCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGAATTCCCGGTGTAGCAGTGAAA
TGCGTAGAGATCGGGAGGAACATCCATGGCGAAGCGAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACG
CAGTATCGAAGCTAACGCGTTAAGTTCGCCGCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATGTCGAGAACT
TCCAGAGATGGATTGGTGCCTTCGGGAACCTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGAAAAAANTT
TGG***NN
NN
NN
NN
N

Endophyte: 52

GGTGACGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCT
CAACCTGGGAATGCATTCAAACTGTGAGCTAGAGTATGGTAGAGGGTGGTGAATTTCTGTGTAGCGGTGAAAT
GCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACTGGACTGATACTGACACTGAGGTGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTA
GTGGCGCAGCTAACGCATTAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGG
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACCT
TCCAGAGATGGATTGGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCTGAGATGTTG
GGTTAAGTCCCGTAACGAGCGCAACCCTTGTCTTGTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGT
CGGTACAGAGGGTTGCCAAGCCGCGANGTGGAGCTAATCCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACT
CGACTGCGTGAAGTCGGAAT

Endophyte: 53

GAGGGTGCAAGCGTTAATCGGATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGG
GCTCAACCTGGGAATGCATTCAAACTGTGAGCTAGAGTATGGTAGAGGGTGGTGAATTTCTGTGTAGCGGTGA
AATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACTGGACTGATACTGACACTGAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTC
TTAGTTGGCGCAGCTAACGCATTAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAA
CTTTTCCAGATGGATTGGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGAAAAA*
**NNNNNTTNGNN
NN
NN
NNNTNN

Endophyte: 54

CTANGTGTGCANGNGGNNNATGCGGTAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTNGTTAAGTNNNGATGTGAAAG
CCCGGGCTCAACCTGGGAATGCATTCAATACTGTCCAGCTAGAGTATGGTAGAGGGTGGTGAATTTCTGTGTAGC
GGTGAATGCGTAGATATAGGAAGGAACACCNNTGGCGAAGGCNACCACCTGGACTGATACTGACACTGAGGNGCGA
AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGNCAACTNGCCGTTGGGAGCCTTG
AGCTNTTAATGGNCCAACTTACCNTTAANTTNACCGNCTNNGGANTACCGGCNCNAGGATNAACTNAAATGAATTG
NCCGGGGCCNCACNANCNGGGGANCATNNGGNTTAATTNCAANCNACCCAAAAACCTTACCNNGNCTTGACNTTCA
ATGAACNTTNCAAAAANNGATTGGNGCCTTNNGGAAACNTTGAACNNGNNGCTNNATNGNTNNTCAACTTNTGTGCA
AAAANATNTTGG***NN
NN
NN
NN

Endophyte: 55

AAGGTGCAAGCGTTACTCGGATTACTGGGCGTAAAGCGTGCGTAGGTG
GTGGTTTAAAGTCTGTTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCA
GTGGATACTGGATCACTAGAGTGTGGTAGAGGGTGGCGGAATTCCCG
GTGGATACTGGATCACTAGAGTGTGGTAGAGGGTGGCGGAATTCCCGGTGGATACTGGATCACTAGAGTGTGGTAGAG
GGTGGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCGTGGCGAAGGCGGCCACCTGGG
CCAACACTGACACTGAGGACGAAAGCGTGGGGAGCAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATG
CGAACTGGATGTTGGGTTCAACTTGGAAACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCTGGGGAGTACGGTCGC
AAGACTGAACTCAAAGGAATTGACGGGGGGCCGACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGA
ACCTTACCTGGTCTTGACATCCACGGAACCTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCAT
GGCTGCTGCAGCTCGT***NNAAAAAANNTTNGNN
NN
NN
NN

Endophyte: 56

GCTCNGTCGTGANANGTTGANNCAATTCCGGGGCGNANNGANCTCGTNCGCGGANTGTNGCGTCTGCTGTGAAATCCCCG
AGGCTCAACCTCGGGCTTGACANTGGGTACGGGCAGACTAGAGTGCGGTAGGGGANATTGGAATTCCTGGTGTANCCGG
TGGAAATGCCGCATATATCAGGAGGAACACCTGATGGCGGAAGGCAGATCTCTGGGCGCTAACNTGANGCTGANGANCN
GAAATCNTGGGGAGCGAANANGATTATATNCNCNTGGTTNTCCANGCCGTNAACNGCNGGGNGCTANATGTANGGAC
CCTTNCNTNGAATTCTGTNTCNGTANCTNACGTATTACATTCCCTCCCTTCCCTNNGNANTACCNGNNNNNNATNTTNTATAN
NCTNNNNNGANTTGNCGGTNGNCTCNCNCNACCAGCNCNTCTATGCTGNNTNCTNTNNCTNNTATCCCATAAATNANC
NNNACNATTGNTTGACTTTACANATNNAANCNNGCCANAGNANGGATCANTNTNTNTATGTTNNTNNTNCTATNNNC
TGCGNCGCNCNGNNCNAACAGNNCNAACANNNGNTNACNCTNNAGCNCNCTNNCNCNNGGCNCCNCTCNCCTCAC
NTNNNNANNNNCTCANACGNTNATNANNTANTTNTNANNNANNAATTTTCNNTNNCNCAATTTTACNNNTTCTTTTCNC
GTTGCNANNCNNGNNNGATTATNANTAATNNNNNTNTNTTACCTTATCNCNCNCTNNGNNGCNGNNGNAGACNNACTNN
NNNNNGTTCNNCNCNCGNTTANNNATTNATNANAAANNATATTACNANGNTCGAATNANNCNNAATNTNATTAACNC
NNCCATANNCNCCNCTNNNTTTTTTTT

Endophyte: 57

AAGGTGCAAGCGTTCTCGGATTACTGGGCGTAAAGCGTGCCTAGGTGGTTGTTAAGTCTGTTGTGAAAGCCCTGGGCT
CAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGAATTCCCGGTGTAGCAGTGAAAT
GCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGC
AGTATCGAAGCTAACGCGTTAAGTTCGCCGCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGG
CCGCGACAAGCGGTGGAGTATGTGGTTAATTTCGATGCAACGCGAAGAACCCTTACCTGGTCTTGACATGTCGAGAACTT
TCCAGAGATGGATTGGTGCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTGAGCTCGTGTGCAAAAAANNTT
GG***NN
NN
NN
NN

Endophyte: 58

GTGCNAAACAAGGTAGCCGTAANCTTGGGTCCCGGNGNCACGTAGGCGGATATTTAAGTCAGGGGTGAAATCCCGCAG
CTCAACTGCGGAAGTGCCTTTGATACTGGGTATCTTGAGTATGGAAGAGGTAAGTGGAATTCCGAGTGTAGAGGTGAA
ATTCGTAGATATTCGGAGGAACACCACTGGCGAAGGCNNGCTTACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGT
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAATGTTAGCCGTCGGGCAGTATACTGTT
GGTGGCGCAGCTAACGCATTAAACATTCGCTACGGGAGTACGGTTCGCAAGATTAAAACTCAAAGGAATTGACGGGGG
CCCGCACAAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGCAGAACCCTTACCAGCTCTTGACATTGGGGGTATG
GGCATTGGAGACGATGTCCTTCAGTTAGGCTGGCCCCAGAACAGGTGCTGCATGGCTGTCGTGAGCTCGTGTCTGTGAGA
TGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTCGCCCTAGTTGCCAGCATTTAGTTGGGCACTCTAAAGGGGACTGC
CGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTCAAGTCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACA
ATGGTGGTGACAGTGGGCAGCGAGACAGCGATGTGAGCTAATCTCCAAAAGCCATCTCAGTTCCGATTGCACTCTGC
AACTCGAGTGCATGAAGTTGGA

Endophyte: 59

AGGTGCAAGCGTTCTCGGATTACTGGGCGTAAAGCGTGCCTAGGTGGTTGTTAAGTCTGTTGTGAAAGCCCTGGGCTC
AACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGAATTCCCGGTGTAGCAGTGAAATG
CGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGGA
GTATCGAAGCTAACGCGTTAAGTTCGCCGCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGG
CCGCACAAGCGGTGGAGTATGTGGTTTAAATTCGATGCAACGCGAAGAACCCTTACCTGGTCTTGACATGTGAGAACTTT
CCAGAGATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTGAGCTCGT***NGNNGAAANN
TTTGGNN
NN
NN
NN

Endophyte: 60

GGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGAGGCGGTCTGTTAAGTCAGATGTGAAATCCCC
GGGCTTAACCTGGGAAGTGCATTTGAAACTGGCAGGCTTGAAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGAGATCTGGAGGAATACCNNGTGGCGAAGGCNNGGCCCTGGACGAAGACTGACGCTCAGGTGCGGAA
AGCGTGGGGAAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGATGTCGAGCTGGAGGTTGTCCTTGA
GGAGTGGCTTCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGA
CGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAAATTCGATGCAACGCGAAGAACCCTTACCTACTCTTGACATCCAG
AGAACTTAGCAGAGATGCTTTGGTGCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTGAGCTGTGTGNAAAA
AA***NNNTTGGNN
NN
NN
NN

Endophyte: 61

CGNTCTGTCGTGAAANGTTGACNCATCTCCGNNGNANNAGACCTCNTNCGCGGNATGTTGCNTCTGCTGTGAAATCCCG
AGGCTCAACCTCGGGTCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCAC
GGATTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATA
NAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTNTGA
NATGTTGGTNTTTT***NNNTCGACNCTNNCANCNTTNNCNCCTNCGCCCCCTTNNNGNNATANNANTCNNCTCNNNNCNT
ACCCCCNNNTNTNCGATTTCCCCCCCCCNCNNNTNCCTCNCNTACNNTTCCCANCTANNCCCTNTTTCNCCCCCNTNN
CCCGNTTNNNNCNTGNAATTNNNTNNCNCNCNTATANTAGTNNNATTNATNTTGTCTGGNTCCCTNTCTCACNNCNC
NNCNTNTTNCNTTACCCNNNNCNCNTCNCNATTAANTNTANT

Endophyte: 62

CGCTCNGTCGTGAGANGTTGNGTCATTATTGGGCGTAAAGGAGCTCGTNTGCGGATNGTNGNGTCTGCTGTGAAATCCC
CAGGCTCAACCTCGGGTCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGG
TGGAATGCGCAGATATCAGGAGGAACACCGATGGCNGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGGAGCGAA
AGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTANTTGTGGGGACCATTC
ACGGTTTCCGTGACGCAGCTAACGCATTAANTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATT
GACGGGGACCCGCACAAGCGGCGGAGCATGCTGGATTAATTCNATGCAACGCGAAGAACCTTACCAAGGCTTGACATA
TACNAGAACGGGCCAGAAATGGTCAACTCNTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTG
TGAGAATNGCNGGNCCAAT***CCCCCTCCNNNTTNTCNTTANTATANTTNNCNCNNNNCNCNNNTANNCGNNNTNAT
NNATNCCCCNNNNCNCNTNNCCTTTCCNNCNTTTNNNGNAGANCCCCCTAAANNCCNCCTTTNATTNCANCNNNAATTN
CTCCNNNANNTANTNNNTTANCAATNTTNNNNCTGCNCNACNANTCCNNNCNCNTTNTNACTCNCNCNCANNATNCC
NNCTATTNTTATT

Endophyte: 63

GGAGGTGCAGCGTTAATCGGATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAATGCAATTCGAAACTGCGAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCNNGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCACTTGGAGGTTGTGCCCTTGAGGCG
TGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCGCACAAGCGTGGAGCATGTGGTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAA
CTTNCCAGAGATGNNTTGGTGCCTTCGGGAACCTCTGAGACAGGTGCTGCATGGCTGTCTGTCAGCTCGTGTGTGAAATG
TTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTATCCTTTGTTGCCAGCGGTNCGGCCGGGAACCTCAAAGGAGACTGCC
AGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGTACAAT
GGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCCTGCTAGTCCGGATTGGAGTCTGCA
ACTCGACTCAT

Endophyte: 64

AGTACTNACANGTNGCCGNAAGCTNTTGTCTCNCGGAAANGGCAACATNGGCGGN***CTTTTAAAGTCGGAGGTGAA
AGCCACAGGCTCAACCCTGGAATTGCCTTCGATACTGGGAGTCTTGTAGTTCGGAAGAGGTTGGTGGAACTGCGAGTGT
AGAGGTGAAATTCGTAGATATTCGCAAGAACACCGGTGGCGAAGGCNNGCCAACTGGTCCGAAACTGACGCTGAGGC
GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGGGA
GCTTGCTCTTCACTGGCGCAGCTAACGCTTTAAGCATTCGCGCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAA
TTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTAATTNNAAGCAACGCGCAGAACCTTACCAGCTTTTGACAT
GTCCGGTTTGATCGGCAGAGATGCCTTTCTTCACTTGGCTGGCCGGAACACAGGTGCTGTCATGGCTGTCTGACGCTCG
TGTCGNGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTCGCCCCCTAGTTGCCATCATTACGTTTGGGAACTC
TTAGGGGACTGCCGTGATAACCCGCCNAGAAGGTGGGGGATGACGTCAAGTCCCTNATGGCCCTTACAGGCTGG***
NGCTNCACACNTGCTACAATGGCGGNGACAATGGGCANNNNNAGGGNGANCTCNANCCTAATTCCAANAGCCCCCTC
ANTNNAAATTGCATTCCNNCTCNCNTTGCATT

Endophyte: 65

GCTCNGTCGTGAGGAGTTGCGGTATTATTGGGCGTAAAGGAGCTCGTANGCGGTTTGTCTGCGTCTGCTGTGAAATCCCG
AGGCTCAACCTCGGGTCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCNGATGGCGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGGAGCGGAAA
GGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCA
CGGATTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTG
ACGGGGACCGCACAAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATA
CGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTCGGA
AAATGTTTG***NN
NN
NN
NN

Endophyte: 66

TGGCTCTGTCGNGAAN***GTTGACNTTTTGGGCGTAAANAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGA
GGCTCAACCTCGGGTCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTG
GAATGCCGAGATACAGGAGGAACACNGATGGCGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGGTTGGGAACTAGTTGTGGGGTCCATTCCA
CGGATTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTG
ACGGGACCCGCAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCTGAAGAACCCTACCAAGGCTTGACATAT
ACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGNCAGCTCGTGTCTGCT*
**NAAATGTTGANCACNTNNTNTNANTCNGCNCNCCCTTTTCNGCNCNGTNCNNANATCATTNTANTTATNANNTNTG
NNCCTACNACCNCCTTTCTGTNGTTCNTCTTNCNTNCCCTATTTCCCTNATGCTNATNCNNCCNTNCCNNGATATTAN
NANCNTNATTTCTNTNTNTNTCTTTATNTNATCNCNCAATNTANATAACCCCNCAANNANNTTNCCCGCATNN
NNCANCNTNATNNCANN

Endophyte: 67

CNNGGTGTCNGNGNN***TGCGGATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTAAGTCTGTTGTGAAAGCCCTG
GGCTCAACCTCGGGTCTGCAGTGGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGAATTCGGGTGTAGCAGTG
AAATGCCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGC
ACGCAGTATCGAAGCTAACGCGTTAAGTTGCGCCGCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCCTACCTGGTCTTGACATGTCGAG
AACTTTCCAGAGATGGATTGGTGCCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGAAAA
AAGGTGG***NN
NN
NN
NN

Endophyte: 68

GGAGGTGCAAGCGTTAATCGGATTACTGGGCGTAAAGCGCACGCGAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGG
GCTCAACCTGGGAATTCGAAACTGCGAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGA
AATGCCGTAGAGATCTGGAGGAATACCNGGTGGCGAAGGCGGCCCTTGACAAAGACTGACGCTCAGGTGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGC
GTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCCTACCTACTCTTGACATCCAGAGA
ACTTTCCAGAGATGNNTTGGTGCCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGTGAAAT
GTTGGGTAAAGTCCCAGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTNCGGCCGGAACTCAAAGGAGACTGC
CAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACAGGTGCTACAA
TGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCNGATTGGAGTCTGC
ACT***N

Endophyte: 69

GGNGCTGNNGGNNGNTCGNN***ATTACTGGGCGTAAAGGGAGCGTAGGCGGACATTTAAGTCAGGGGTGAAATCCCCG
GGGCTCAACCTCGGAATTCGCTTTGATACTGGGTGTCTTGAGTATGAGAGAGGTGTGTGGAATCCGAGTGTAGAGGTG
AAATTCGTAGATATTCGGAAGAACACCACTGGCGAAGGCGACACTGGCTCATTACTGACGCTGAGGCTCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGTAGTTGTGCGGGATGCATGCATTC
GGTGACGCAGCTAACGCATTAAGCAATCCGCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGG
CCGCGACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCCTACCACCTTTTGACATGCCTGGACCGC
CAGAGAGATCTGGCTTTCCCTTCGGGGACTAGGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCTGAGATGTTG
GGTTAAGTCCCAGCAACGAGCGCAACCCTCGCCATTAGTTGCCATCATTTAGTTGGGAACTCTAATGGGACTGCCGGTGC
TAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACAGGGTGGGCTACACAGGTGCTACAATGGCGA
CTACAGAGGGTTAATCCTTAAAGTCGTCTCAGTTCCGATTGTCTCTGCAACTCGAGGGCATGAAGTTGGAATCGCTA
GTAATCGCGG

Endophyte: 70

CGCTCNGTCTGAGNGTTGANTNTTTTTGGGCGTAAAGGAGCTCGTANGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCCGAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAAGTACGCTGAGGAGCGAAAGGG
TGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCACCG
TTTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGACCCGCAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCCTACCAAGGCTTGACATATACGA
GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCTGAGG
ATGTTTGA***NNCANNNTNCCGTNANNCCCAATNTTCNANATCNCNNCANNNCANATTTNTCNCNCNCANTCCC
CCTTCNNCATTTTTNTNTNTNTNNCTTNNNTCTCCANANNNCNCTANCCNGCAAATCNCNCNTTNNNNCNTNTTN
GCNANTANNNTNATNCCNNCNNTNCANTNCNTTCCATCNCNNCNCCCTNCTNCTNCCNCCCNTNTTTTNNCNCNC
NCGTTCCCNCTNTTTNTNTC

Endophyte: 71

GTNTACNCGAGTNNNNCCCNATGNNCGTNNNTNTNGCGGAAATACTNNTNGGCGGCTTGTCGCTGTCTGCTGNGAAATC
CCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGC
GGTGGAAATGCTGCAGATATCAGGAGGAACACCGATGGCAGAAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAG
CGAAAGGGTGGGGAGCAGNNNGGCTTANATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCA
TTCCACNGTTTCNTGACGCANCTCAGCATTAAATTTCCCGCCTGGGGANTNCNGTCNTAANGCTAATACTCANAG
GAATTGACNGGGACCCNCACAACCGGGCGGAGCCATGCGNATCAATTTGAATGCANCGCANNANCAACCTTACCAAGGCC
TTGNCATNTNCNACACCGNCCACAAAANGGTCAACTCTTTNNACACTTCTANANACAGGNGNNTGCNTNNNCTNANNN
TAGCCNCCCTNATNTTGA AAAANGTTACNCNNNAANNCCNCCCATCNCAGCNC AAAANCCTCCTANCATAATNTTANC***
NNCNGTNTNGTNTNTAANCCTTNNNGNCACTNCTNNCNGNCCACTCCTNCNGANCCTNNAGTTTGANCNNCAAATA
CNCATGCGNCTTNTTGTCCNANTNCCNNAACCCCTNGNNTNAAATACCCCNNTCTANAATGACNTGNNNNNTCTNT
NTAGNNTGANCNNCATAACNNAATAGNCNTTANNANCNCTCNGNTTAAAGGTTTACNCNCACTCTCNCCACTT

Endophyte: 72

ANCGACNNGNNNNCGTAAGGTTNGGTTCCGGGAAANCGTNCNCANGCGGTGATGTAAGACAGTTGTGAAATCCCCGG
GAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCGCGTGTANCACTGA
AATGCGTAGATATGCTGGAGGAACACCGATGGCGGAAGGCNATCCCTGGGCCTGTACTGACGCTCATGCACNAAAGC
GTGGGGAGCAAANGGGATTAGATACCCTGGTANTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGNCT
CAGTTNCGAAGCTAACNCNTGAAGNTGACCCCTNNGGAGTACGNGCGCAAGGGTGA AAACCTCNAANGAATTGACGGG
GACCCCAACAAGCGGNGGATGATGTGGTTAATNTCATNCAACGCCGAAAAACCTTNCACCTNTGNCATNCNCGN
NNTNTTNCNGNCNATGGCTTANTGCTTCGAAAGACANCCNGGACNCCACGNCNNTNCATGGCCCTGCCNTGAAGGTCTN
CCNTACGNGAGATTGTTGGGTAAANTCCNGNACNGACNGCAACCCCTNGTCTATTATNTCCTTACA***NNNAGCTNNCG
GCGACTCANNNTTCNCAACNGNNNCGAACNNACGNGNTCGAAGGGGCGGCGNNGCNGACAGGNCCTCNTCGGGCCNN
NNNAAGTTGGGGGNTNNNNCCNNAACANNCTNNGCCTNGTNCNATACGGNNNCNCAACCCNNGNNGGTGNNAACNN
CTNNANTNTNNNGCNGGNCNTNANNNCNCAANCANTNGNTNCCNGCNCNT

Endophyte: 73

CGCTCNGTCGTGAGNGTTGNNN***ATTATTGGGCGTAAAGGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCC
GAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCTG
GTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAA
AGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTC
ACGGTTTCCGTGACGAGCTAACGCATTAAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATT
GACGGGGACCCGCAAAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATAT
ACGAGAACCGGGCCANAATGGTCAACTTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTGACGCTCGT***
NTGNAANGTTGCCNACCTNCNNCNCNCCNCTCNTNTGTTNNNCTNNTCACCACCTNNANCNNNTCNCNTTNTNCANTN
NCANNNTCNTTTNATNNNTCACNNNATNCTCCTNCNCTCNCNTTCCCTCCNCCCTCCNNNNCCNCCACTCCCCCNCN
NNTNCCNCTTCTCNCNCCCTANNACCTCCNCCCTNCTANTCCNNNTATNNACNNNATANNNTTCCNCCNCTCCCC
NNNCCNCCNATNTNTNTNTT

Endophyte: 74

GTCNACAAGGTAGCCGTAAGCTTGGTTCCCGGGTAAAGAGNTCGCAGGCGGTTANTTAAGACATGATCGTGAAATCCC
GAGGCTCAACCTCGGGCCTGCANTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGC
AGGTGAAATGCGNAGATATCCGGAGGAACACCCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGATGCGCG
AAAGGGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACCCCNATAACGATGNGAACTAGTTGTGGGGNCNNTA
CTANNNNATNNGTGACGCTNACTACCNCANTNNGACCCCTCCTGNGGANTACNGCCGNCNNGGANANTACNNAANG
AANCAGCGNNNNCCCNACNAGCGGANGATCNTGCTGATGNATATGNNNGCAACNNNAANANNNNNNCCNTNGCNCGT
CTTNTACANNATCNGGNCATAAAATGGTNAANTCTNNGNANACNCGNACAGGTGGTGCATGGNTGTCGTGACGCTCGT
GTCGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTNGTNTATGTTGCNANCACNTAANGGTGNNANCTCA
NGGGATACTGCCGGGGTCAACNNGGAGGAAGGTGGGGATGACGCNNANTCATCNTGCNCCTTATGTTNNGNGCTTCAN
NCATGCTANANTGGCCGGNACNAANGGCTGCNATACCGGGANGTGNANCGAATCCCAAANAGCCGGCCCCATCTCNG
ATTGANGNTGCNCTCGA

Endophyte: 75

TCNACACGGTAGCCGTAAGCTTNGTTNCCGGGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCGAGG
CTCAACCTCGGGCCTGCANTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
ATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGANGAGCGAAAGGGT
GGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCACGGT
TTCCGTGACGCAGCTAACGCATTAAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGACCGCACAAAGCGGCGGAGCATGCNGATTAATTCGATGCAACGCGAANAACCTTACCAAGGCTTNNCATATACCAT
AACGGGCCAGAAATGNTCANCNTCTNTGGACACNCTNTAAACAGGTGGTGCATGGNTGTCNTCAACNTCGTGTCTGGA
AGATGTTGGGGTTTAAAGTNTCCGAACGAANCAGCAACCCCTTGTNTNTATGTTTGCCACCCCTAATGGGTGGGAAC
TNCATGNNGATACTNACGGGGTGCNCNTCNGA***NNNGAAGGTNNNNAATNACCCCTNNATCNCATNCCCCCTTAA
TCTCTANNGCTNTCACTCATANCTNNAATGGCAGCTNNANAAGGCNNCANCCNCCGATGNNNGANNNAATCCCNAA
ACANACGNNCCNCTCCNNAT

CNCAAGCGTTATCCGGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAGGCTCAA
CCTCGGGCCTGCAAGTGGGTACGGGCAGACTAGAGTGCAGTAGGGGAGATTGGAATCCTGGTGTAGCGGTGGAATGCG
CAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAGGGTGGGGA
GCAAAACAGGCTTAGATACCTGGTAGTCCACCCGTAAACGTTGGGAACCTAGTTGTGGGGACCATTCACGCTTTCGGT
GACGCAGCTAACCGCATTAAGTTCCCGCCTGGGGAGTAGCGGCCGCAAGGCTAAAACTCAAAGGAATTGACGCGGGACCC
GCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAACGGG
CCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTCTGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCTCTGTTCTATGTTGCCAGCACGTAATGGTGGGAACCTCATGGGATACTGCCGGGGTC
AACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTTGGGCTTCACGCATGCTACAATGGCCGGT
ACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCAAAAAGCCGGTCCCAGTTTCGATTGAGGTCTGCACCTGCACC
TCATGA

TAGGCGCAGCGTTTCCGGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAGGCTC
AACCTCGGGGCTGCAAGTGGGTACGGGCGAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATG
CGCAGATATCAGGAGGAACCCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGTGGG
GAGCAATAACAGGCTTAGATACCTTGGTAGTCCACCCCGTAAACGTTGGGAAGTGTGTGGGGACCATCCACGGTTTCC
GTGACGCAGCTAACGCATTAAAGTTCCCGCGCTGGGGAGTACGCGCGCAAGGCTAAAACTCAAAGGAATTGACGGGGAC
CCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAACG
GGCAGAAATTGGTCAACTCTTTGGACACTGCTAAACAGGTGGTGCATGGTTGTCGTCAGCTCTGTGTCGTGAGATGTTGG
TTAAGTCCCGCAACGAGCGCAACCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAAGCTCATGGGATACTGCCGGGG
GCTAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTATGTCTTGGGCTTACGCATGCTACAATGGCCG
GTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCAGTTCGGATTG

[illegible][illegible]

CGCTCNGTCGTGAGNTGTTGCN***TATTATTGGGCGTAAAGAGCTCGTANGCGGNTNGTCGCGTCTGCTGTGAAATCCC
GAGGCTCAACCTCGGGTCTGCACTGGGTACGGGACAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGG
TGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCGAGATCTCTGGGCCGTACTGACGCTGAGGAGCGAAA
GGGTGGGAGCAACACAGGCTTAGATACCTGGTAGTCCACCCGTAACAGCTTGGGAATAGTTGGGGTCCATTCCA
CGGATTCGTGACGCGACTAACCGCATTAAAGTTCCCGCTGGGGAGTACGCGCGCAAGGCTAAAGCTCAAAGGAATTG
ACGGGGACCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGC AACGCGAAGAACCTTACCAAGGCTTGACATATA
CGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGNA
GAATGTTGG***NNNNNNNNNNNNNNNTNNNNNNNNCNN
NNNNNNNNNNCNNNNNNNNNNNNNNNNNNNNNTNNNTNNNNNTNNNNNNNNNNNNNNCCNNNNNNNNNNNNNN
NNNNNNNNNTNNNNNNNNNNNNNNNNNNNNNTNNNTNNNNNNNNNNNNNNNNNTNCCNNNNACNNNNCNCNNN
CCNNTNNNNNT

Endophyte: 81

CTCGGTCCNGAGATGTTGCGGATTATTGGGCGTAAAGGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAGGG
TGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCACGG
TTTCCGTGACGCAGCTAACGCATTAAGTTCCTCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGA
GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTGTCGTACGCTCGTGTCTGNA
ATGTTG***NN
NN
NN
NN

Endophyte: 84

AAGGGGCTGCNTNGNTCGGATCACTGGGCGTAAAGGGCGCGTAGGCGGACTTTTAAGTCGGAGGTGAAAGCCCAGGG
CTCAACCTGGGAAGTGCATCTGTGACTGCATTGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGAGGTGAA
ATTCTGTAGATATTCGCAAGAACACCGGTGGCGAAGGCNGGCCAACTGGTCCGAAACTGACGCTGAGGCGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTGGGGAGCTTGCTCTTC
AGTGGCGCAGCTAACGCTTTAAGCATTCCGCGCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTGAAGCAACGCGCAGAACCTTACCAGCTTTTGACATGTCGGGTTTGA
TCGGCAGAGATGCCTTTCTTCAGTTCGGCTGGCCGGAACACAGGTGCTGCATGGCTGTCGTACGCTCGTGTGCGGAAAT
***NTNTTNGNNCCNCNNNNNNNCNTTNNCNCNTGTTNNCNGNGNNNGNCNGNNCNGGANANGNGCTTNNNCATNTN
ANNNNTNTNTTNNGCNNAANCCNCNNNTTNNNNAANNCANAACNNNNNTNTCTCNNACTACNGNNTTNNCNCNN
TCNNCCCTAGNNTCNCNCNNNTTNNNNCNCNACNNANNGNNTTCNNNNNCCATNNAANNNNNCCCCNTCT
NTNNCCNNTNTNCTCTACCNANCCNCANN

Endophyte: 85

TAGGGTGCAGCGTTAATCGGATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGTTGTGAAATCCCCGGG
CTCAACCTGGGAAGTGCATCTGTGACTGCATTGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGAA
ATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCTGTACTGACGCTCATGCACGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAG
TAACGAAGCTAACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGAC
CCGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCACCTTTGACATGTACGGAATTTG
CCAGAGATGGCTTAGTGCTCGAAAGAGAGCCGTAACACAGGTGCTGCATGGCTGTCGTACGCTCGTGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTATTAGTTGCTACATTAGTTGGGCACTCTAATGAGACTGCCGGTGA
CAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCCTATAGGTGGGGCTACACACGTCATACAATGGCTG
GTACAAAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCATAAACCAGTCGTAGTCCGGATCGCAGTCTGCAACTC**
*N

Endophyte: 86

TAGGGTGCAGCGTTAATCGGATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGTTGTGAAATCCCCGG
GCTCAACCTGGGAAGTGCATCTGTGACTGCATTGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGAA
AATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCTGTACTGACGCTCATGCACGAAAGCGTG
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCA
GTAACGAAGCTAACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGA
CCGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCACCTTTGACATGTGCGGAATTT
GCCAGAGATGGCTTAGTGCTCGAAAGAGAGCCGTAACACAGGTGCTGCATGGCTGTCGTACGCTCGTGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTATTAGTTGCTACATTAGTTGGGCACTCTAATGAGACTGCCGGT
GACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCCTATAGGTGGGGCTACACACGTCATACAATGGCTG
TGGTACAAAGGGGTTGCCAACCCNCNANGGGGAGCTAATCCCATAAACCANTCGTANTCCGGATCG

Endophyte: 87

GTGGCAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCACGGCTC
AACCCTGGAGGGTCATTGGAAACTGGAAACTTGAGTGCAGAAGAGGAAAGTGAATTCATGTGTAGCGGTGAAAT
GCGCAGAGATATGGAGGAACACAGTGGCGAAGGCGACTTTCTGGTCTGTAAGTACGCTGATGTGCGAAAGCGTGGG
GATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTTCCGCCCTTAG
TGCTGCAGCTAACGCATTAAGCACTCCGCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACC
CGACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAATCTTGACATCCTCTGACCCCTC
TAGAGATAGAGTTTTTCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTGTCGTACGCTCGTGTGCGGAAANN
TGG***NN
NN
NN
NN

Endophyte: 88

CTCGGTNTGAGNGTTGCGGATTATTGGGCGTAAAGCGCGCGTAGGGCGTTTTTTAAGTCTGATGTGAAAGCCACGGCT
CAACCGTGGAGGGTCATTGGAAGTGGAAACTTGAGTGCAGAAAGAGGAAAGTGGAAATTCATGTGTAGCGGTGAAAT
GCGCAGAGATATGGAGGAACACCAAGTGGCGAAGGCGACTTTCTGGTCTGTAACAGCTGATGTGCGAAAGCGTGGG
GATCAACAGGATTAGATACCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCTTAG
TGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACC
CGCACAAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTCTGACCCTC
TAGAGATAGAGTTTTCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGGAAAAAANT
TG***NN
NN
NN
NNNNNNNNNN

Endophyte: 89

CCTCNGCTGAGNGTTGCGNATTATTGGGCGTAAAGGAGCTCGTANGCGGATNGTCGCGTCTGCTGTGAAATCCCGA
GGCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTG
GAATGCCGAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGACGCTGAGGAGCGAAAGG
GTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACG
GATTCCGTGACGCAGCTAACGCATTAAGTTCCCGCGCTGGGGAGTACGGCCGCAAGGCTAAAGTCAAAGGAATTGAC
GGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACG
AGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCTG***N
NNATGTTGNCNN
NN
NN
NANNNNNNN

Endophyte: 90

AAGGGGCTGCNTNGCTCGGATTACTGGGCGTAAAGGGAGCGTAGGGCGACATTTAAGTCAGGGGTGAAATCCCGGGGC
TCAACCTCGGAATTGCCTTTGATACTGGGTGCTTTGAGTATGAGAGAGGTGTGTGGAACCTCCGAGTGTAGAGGTGAAAT
TCGTAGATATTCGGAAGAACACCAAGTGGCGAAGGCGACACACTGGCTCATTACTGACGCTGAGGCTCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTGCGGGATGCATGCATTTCGGTG
ACGCAGCTAACGCATTAAGCAATCCGCCTGGGGAGTACGGTGCAGAAAGATTAAGTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCACCTTTTGACATGCCTGGACCGCCAC
GGAGACGTGGCTTTCCCTTCGGGGACTANGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGNANGAAAA***NT
NNGNN
NN
NN
NN
NN

Endophyte: 91

AAGGGGCTGCGTTGCTCGGATTACTGGGCGTAAAGGGAGCGTAGGGCGACATTTAAGTCAGGGGTGAAATCCCGGGGC
GGGGCTCAACCTCGGAACCTGCTTTGATACTGGGTGCTTTGAGTATGAGAGAGGTGTGTGGAACCTCCGAGTGTAGAGGTGAAAT
TCGTAGATATTCGGAAGAACACCAAGTGGCGAAGGCGACACACTGGCTCATTACTGACGCTGAGGCTCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTGCGGGATGCATGCATTTCGGTG
ACGCAGCTAACGCATTAAGCAATCCGCCTGGGGAGTACGGTGCAGAAAGATTAAGTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCACCTTTTGACATGCCTGGACCGCCAC
GGAGACGTGGCTTTCCCTTCGGGGACTAGGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGNAAAAGTNTTG*
**NN
NN
NN
NN

Endophyte: 92

CAAAGNTGCTNTCGTNNTACNTN***TACTGGGCGTAAAGCGCACGTAGGGCGACATTTAAGTCAGGGGTGAAATCCC
GGGGCTCAACCTCGGAACCTGCTTTGATACTGGGTGCTTTGAGTGTGGAAGAGGTGAGTGAATTGCGAGTGTAGAGG
TGAAATTCGTAGATATTCGCAGGAACACCAAGTGGCGAAGGCGGCTGACTGGTCCCAACTGACGCTGAGGTGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGTGCGCAAGTTTACT
TGTCGGTGGCGCAGCTAACGCATTAACATTCGCCCTGGGGAGTACGGTGCAGAAAGATTAAGTCAAAGGAATTGACG
GGGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCTACG
ATCGCTACAGAGATGTAGTTTCACTTCGGTGGCGTAGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCGAGAA
AA***NNNTTGGNN
NN
NN
NN

Endophyte: 93

GCTCNGTCTGAGNGTTGANTCTTTNNGGGCGTANN***GGAGCTCGTACGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCCGAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAGGG
TGGGGAGCAAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCACGG
TTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCGCTGGGGAGTACGGCCGCAAGGCTAAACCTCAAAGGAATTGACG
GGGACCCGCACAAGCGGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGA
GAACGGNTCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCTGNANA
TGTGT***NNNNNNACNCCCCCTNNTNNTNTTNNCCCTNCCNCTCCACNTNTNCCNANANCNNNCCACNGNCNAT
NNTCNANNCNCCNCTNNTNANCTTNTCTNATNTNCCNCCNCCNANTGNCANCCNCCNNTCCNANNCNACNNNAC
NCNATNTCANTCCCNATTNNCCNANNCNCCNACAGACCNAGANTNTTNTAANNGCCNNACNCCTGTNCCNCGCN
NTNCCNANCTTNNNATNTT

Endophyte: 94

GTCNGTCTGAGAGTTGANTCATTNNGGGCGTANN***GGACTCGTAGGCGGTTTGTNGCGTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCCGAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAGGG
TGGGGAGCAAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCACGG
TTTCTGTCGACGCAGCTAACGCATTAAGTTCCCGCGCTGGGGAGTACGGCCGCAAGGCTAAACCTCAAAGGAATTGACG
GGGACCCGCACAAGCGGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGA
GAACGGGGCCAGAAATGGTCAACTCTTTGGACACTCGTANACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCG***NA
ANTGTGNN
NN
NN
NN

Endophyte: 95

CCTCGTCCNGAGNGTTGCGGCATTATTGGGCGTAAAGAGCTCGTANGCGGNTTGTGCGCTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCNGGTG
GAATGCCGAGATATCAGGAGGAACACCGGATTGGCCGAAAGGCAGATCTCTGGGCCGTAACGTGACNCTGNAG
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CCNNTTCCNCGGGTTNCCCGTGNNCCCANCTTAACCCCNNTAAATTTCCCCCCCCCTNNGGGGGANTTNNCGGGCC
CCNNAAGGGGTTTAACTNTNAAAAAGGGANAATTNNGCCGGGGGAACCCCNCCNNNNAAACNCCC***NN
NN
NNNTTNTNTNCCNAAANNNNNNNNNNCCNNNNNNNNANAANGGCGNNGGNNNAGGGGNNNNNANTGGGGAANGGA
GGNTGNGGGGNNNNNTNGGGGNTNNNNNNNNNTATATAANAATTTNNNNNNCNCNNGNCTCNNNNNNNNNNNNNN
NNNNNNCCNNN
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CNNAANNNNNNN

Endophyte: 96

NN***AGGGGCTNACATTGTNCGGATTACTGGGCGTAAAGCGCACGTAGGCGGCTTTGTAAAGTTAGAGGTGAAAGCCTG
GAGCTCAACTCCAGAAGTGCCTTTAAGACTGCATCGCTGAATCCAGGAGAGGTGAGTGGAAATTCAGAGTGTAGAGGT
GAAATTCGTAGATATTCGGAAGAACACAGTGGCGAAGGCGGCTACTGGACTGGTATTGACGCTGAGGTGCGAAAGC
GTGGGGAGCAAAACAGGATTAGATACCCTGGTAGTCCACCGCGTAAACGATGATAACTAGCTGTCCGGGCACTTGGTGC
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CCTTNTNNNN

Endophyte: 97

CGCTCNGTCTGAGNTGTTGCNNCATTATTGGGCGTAAAGGAGCTCGTANGCGGNTTGTGCGCTCTGCTGTGAAATCCC
GAGGCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGG
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GACGGGGACCCGCACAAGCGGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTTGACATA
TACGAGAACGGGCCANAAATGGTCAACTCTTTGGGACACCTCCGTAAACAGGTTGGTGCATGGTTGTGCTCANTCGN
GTCGTGAGAATNTTTGAGNTGNTAA***NNNNGCCTTNTCCNCTCNANTCTCCNNANTNTNTNTNTNTNTATNNNT
CNNTNNNNCNTCTNCCCNNTTTNCCGATNTTATNCTCCNCCCTCTNANCNANNNGCNNNTCATNCGNCTATTNNNG
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CCNCACTNTCNNTCTGNNTNTNTNTNTTCCCCCNNTNNNNCCNCTNCTNNNN

Endophyte: 98

TNCGCTCNGTCTGGAGNGTTNGCGGNATTATTGGGCGTAAAGGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAAT
CCCCAGGCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAG
CGGTGGAATGCGCAGATATCAGGAGGAACACCNATGGCGAAGGCAGATCTCTGGGCCGTAACNGANNNGCTTGAGG
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GACCATTCTNNGGTTTCCGTGACCACNTACNCNNTNAAGTTTCCCCGCTTGGGGGAANTCCGGGGCCCCNAAGGGCTTA
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NGGGTTTNAATTTCCNANGNGCNCNCCCGCAAAAAANNCNCTTNCCTTTCGCAAGGGTTTGGACAATTTTCCAAAAAAN
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CTNNCCCTNTCNCNCNCTNCCNNTTCCNCCNCCNANTCTATNNNNCGCNATTCNNNNNCNCCNCGCCANTACTT
GCGTANNNTTNTATANTTNNGCNTNNNTTNTTCTTNTNCTGTTNCNTTTTTTCNTCCNCATTNNNNNCCNCCCTATT
TTTNNANNCNCNCATNTANNCCNNNNNTNNAC***NN

Endophyte: 99

CTNGNNCTGACGGGTTTGTTCGGCATTACTGGGCGTAAAGGGAGCGTAGGCGGGNCATTTAAGTCAGGGGTGAAATCC
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TGAAATTCGTAGATATTCGGAAGAACACCACTGGCNGAAGGCGACACACTGGCTCATTACTGACGCTTGAGGGCTCGAA
AGCCGTGGGGAGCAAACAGGATTAGATACCCGTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTGCGGGATGCATG
CATTTCCGGTGACGCAGCTAACGCATTAAGCAATCCGCTGGGGAGTACGGTCGCAAGATTAACCTCAAAGGAATTGA
C***GGGGGCCCCACANGCGGGGGAGCATGTTTTTTTTTTNGAAGCANCAGCGCATTACCTTTTTTTTTTTTGACATGCC
CCGGAGGNGGNNGGNNTTTTTTTTTTTTTTTNNANAAAAAANNAAAAAAGGTGCNTGCCCCCTTTNNNGCGGGG
GGGGNGNGGGGGAANATTTTNTCCCCCNCNTTTTTTTTTTTCGGGGGGGNGNGGGGNNANANANNNNNNNNNNNNN
NTTTTTTTNNNGGNGGNN
NN
NN

Endophyte: 100

GTCTAACAGGTAGCCGTAAGCTTGGTACCCGGGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAG
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AATGCGCAGATATCAGGAGGAACACCNATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGG
GTGGGGAGCAAACAGGCTTAGATACCCCTGGTAGTCCACCCCGTAAACGTTGGGAAGTGTGTTGGGGACCATTCACG
GTTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGAC
GGGACCCGCAAGCGGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACG
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GCCGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAATCATCATGCCCTTATGCTTGGGCTTCACGCATGCTACAA
TGCCCGGTACAAAGGGCTGCAATACCCGTGAGGTGAGCGANTCCCCAAAAGCCCGGTCCCAAGTTCGGATTGAGGTC
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Endophyte: 101

GGNNGGGCTACGCTTGTTCGGATTACTGGGCGTAAAGCGCACGTAGGCGGCTTTGTAAGTTAGAGGTGAAAGCCTGGA
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AATTCGTAGATATTCGGAAGAACACCACTGGCGAAGGCNGGCTCACTGGACTGGTATTGACGCTGAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCCTGGTAGTCCACCCCGTAAACGATGATAACTAGCTGTCCGGGCACTTGGTGCT
TGGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGTCGCAAGATTAACCTCAAAGGAATTGACGGG
GGCCTGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGCAGAACCTTACCAAGCTTTGACATGTCCGGTTT
GGTTTCCAGAGATGGATTCTTTCAGTTTCGGCTGGCCGGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGNGAAAAA
AAA***NTTNGGNN
NN
NN
NN

Endophyte: 102

GCTCNGTCCNGAGNGTTGCGGTATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGGG
TGGGGAGCAAACAGGCTTAGATACCCCTGGTAGTCCACCCCGTAAACGTTGGGAAGTGTGTTGGGGTCCATTCCACGG
ATTCCGTGACGCAGCTAACGCATTAAGTTCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACG
GGGACCCGCAAGCGGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGA
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ATTTTGG***NCNN
NN
NN
NN

Endophyte: 103

AAGGNGCTAGCGTTGCTCGGATTACTGGGCGTAAAGGGAGCGTAGGCGGACATTTAAGTCAGGGGTGAAATCCCGGGG
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GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTCGGGATGCATGCATTTCCGT
GACGCAGCTAACGCATTAAGCAATCCGCCTGGGGAGTACGGTCGCAAGATTAACTCAAAAGGAATTGACGGGGGCC
GCACAAGCGGTGGAGCATGTGGTTTAATTGCAAGCAACGCGCAGAACCTTACCACCTTTTGACATGCCTGGACCGCCA
CGGAGACGTGGCTTTCCCTTCGGGGACTAGGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGGANAAAAANTTG
G***NN
NN
NN
NN

Endophyte: 104

GGGGCTAGCGTTGCTCGGATTACTGGGCGTAAAGGGCAGCGTAGGCGGNCATTTAAGTCAGGGGTGAAATCCCGGGG
GGGCTCAACCTGGGAATGCCTTTGATACTGGGTGCTTGAGTATGAGAGAGGTGTGTGGAACCTCCGAGTGTAGAGGTGAAAT
TCGTAGATATTGGGAAGAACACCACTAGTGGCGAAGGCGACACACTGGCTCATTACTGACGCTGAGGCTCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTCGGGATGCNTGCATTTNNGTG
ACGCAGCTNACGCATTAAGCAATCCNCCTGGGGAGTACNGNCGCAAGANTAACTCAAAAGGAATTGACGGGGGCC
NCACAAGCGGNGGAGCATGTGGTTTAATTGCAAGCAACGCGCAGAACCTTACCACCTTTTGACATGCCTGGACCGNCA
GAGAGATCTGGCTTTCCCTTNGGGGACTATGACACANGNGCTGCATGGCTGTCGTCANCTCGTGTGCGTANANGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCCTNNCCATTNNTTGCCATCATTTANTTGGGAACCTCTNATGGGACTGCCGNGCTA
ACCCGAGGAAGGTGGGGATGACGTCAAGTNCCTATGGCCCTTACAGGGNGGGCTACACACGTGCTACNATGGCGACT
ACAGAGGCTNNATCCTTAAAGTGCNTCAGTTCGGATNGTCCTCTGCNACTCGAGGGCATGAAGTTGGAATCGCTAGT
AATCGCG

Endophyte: 105

TAGGGTGCAAGCGTTAATACGGCATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGTTGTGAAATCCCC
GGGCTCAACCTGGGAATGCATCTGTGACTGCTGAGTGTGGAGTACGGCAGAGGGGGATGGAATTCGCGTGTAGCAGT
GAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCTGGGCCTGTACTGACGCTCATGCACGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTACTGACT
CAGTAACGAAGCTAACGCGTGAAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAAGGAATTGACGGG
GACCCGACAAAGCGTGGATGATGTGGTTTAATTGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAAT
TTGCCAGAGATGGCTTAGTGCTCGAAAGAGAGCCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCTGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTGTCTATTAGTTGCTACATTGAGTGGGCACTCTAATGAGACTGCCCG
TGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCTCATGGCCCTTATAGGTGGGGCTACACACGTGCTACATGAGT
CTGGTACAAAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCATAAACCAGTCGTAGTCCGGATCGCAGTCTGCAAC
TCGACTGCGT

Endophyte: 106

CGGTGCANGNNGNCTGGGATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAAGTCTGTTGTGAAAGCCCTGGGCT
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GCGTAGAGATCGGGAGGAACATCCGTGGCGAAGGCGGCCACCTGGGCCAACACTGACACTGAGGCACGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTCAACTTGGAACC
CAGTATCGAAGCTAACGCGTTAAGTTCCGCCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAAGGAATTGACGGG
GCCCGCACAAAGCGGTGGAGTATGTGGTTTAATTGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAT
TTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT***NGAAAAANA
NNTNGNN
NN
NN
NN

Endophyte: 107

CGNTCNGNTCGTGANANGTTGACTCNTGTCGTGANNGTTGANCTCGTGNCGCGGATTGTNGCGTCTGCTGTGAAATCCC
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AAGGGTGGGGAGCAAACANGCTTACATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACCTANTTGTGGGGACCATTC
CACGGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACNGCCGCAAGGCTAAAACTCANAGGAAT
TGACGGGGACCCCTACAAGCGGCGGANCATGCCGATTAATTCATGCAACCNCGAAGAACCTTACCAAGGCTTGACAT
ATACGAGAACCGNCCANAAATGGTCAACCTACTTTGGACACTCCCNAAACANNGNGGNGCNTGGCNGNCACNANCT
NGTGTGCTGANAATGNGTGGCANCCCTTNCNCANNTNACNATNTCTATATNNTNAGANNTGTCCACCNCTCNAN
CNCCCTNNTNTATNTACANCATTNANTNACATNNTCNTNTTNCNCCTANCCANCNCCTTCNCNNCTTTNTNATACA
NTANTNTTGAANANANTATTANNTNCCNNCTNCCTNCTNTTACNTTNNNTACGCCNNCAANNCTCACCCNCT
NNNCCNCCNNCTCCTTCTC

[illegible]

GTCNNACACGGTNGCCGTAGCTTGGCTGNNNGTTAAAGCGTGCGCAGGCGGTGATGTAAGACAGTTGTGAAATCCCCGG
GCTCAACCTGGGAAGTGCATCTGTGACTGCATTGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGA
AATGCGTAGATATGCGGAGGAACACCGATGGCGCAAGGCAATCCCCCTGGGCGCTGTACTGACGCTCATGACGAAAGCGT
GGGGAGCAACACAGGATTAGATACCTGGTATGCTCCAGCCCTAAACAGTATCAACTGTTGTTGGGTCTTCACTGACTCA
GTAACGAAGACTAACCGCTGAAGTTGACGCGCTGGGAGTACGGCCGAAGTTGAAACTCAAAAGGAATTGACGGGGA
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GACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATANGTGGGGCTACACACGTCATACAATGGC
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GAC

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CGTGACGCAGCTAACGCATTAAGTTCCTCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGA
CCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAAC
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GGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACCTCATGGGATACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCC
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CGACCT

[illegible][illegible]

Endophyte: 113

N***CTCNGTCTGAGNGTTGAGNCTTTTGGGCGTAAAGAGCTCGTGTGCGGATANNTNNGTCTGCTGTGAAATCCCGA
GGCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTG
GAATGCCGAGATATCAGGAGGAACACCNATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAG
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GANAACGGGCCAGAAATGGTCAACTCTTTGGACACTCNTAAACAGGTGGTGCATGGNTGTCNTCAGCTCGTGTCCCGA
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CCTANNTANCTCCATTCNATATCTTNTNTNCACNTCCNTNNTC***NNNNNNNNATCCNCCNCCNCCCNAGATTNNN
NTNTNNTTNTNTNCCNGNTNTTCCCNCTTACANNATTCANGNANCTTGANNNCNTNTCCNNANNCACCATANCN
GNCGTCTNNTANNATGTTN

Endophyte: 114

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CTCATGTCTTGGGCTTANNTTAGCTACCAATGG***NNNNNNNTCAAANGGCTTNCANTACCNCCANGTTGCAGC
NANTNCTAAAAANTCTCGTCCCAACTNCGGATNNNGGTNTNANCTNGACCCTATN

Endophyte: 115

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CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTGCGGGATGCATGCAT
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GGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTGCAAGCAACGCGCAGAACCCTTACCACCTTTTGACATGCCTGGA
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NATTTGG***NN
NN
NN
NN

Endophyte: 116

GTCNGTCTGAGAGTTGNGGATTTTGGGCGTAAAGACTCGTANGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAGGCTCA
ACCTCGGGTCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGC
GCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAGGGTGGGG
AGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGATTCCG
TGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGACC
CGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAACCG
GCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCTNAGATGTTGAC
CACC***NN
NNGCTCCTNNTNATNANN
TNCNCCNCCANNTCNACTNTNTNTNCCACCCCTNCNNNNCCNNNTTNNNTNTNTTTN

Endophyte: 117

TGCNCGNNGCNTGAGGTNGTNNCTCGTGTGNGAAAGTTGGCTCGTGTNCGGAAATGTTGGGTCTGCTGTGAAATCCC
GAGGCTCAACCTCGGGCTGCAGTGGGTACGGGCANACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGG
TGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCANATCTCTGGGCCNTAACTGACGCTGAGGANCNAAA
GGGTGGGGAGCNANAGGNTTAGATACCCTGGTAGGTCCACCCCGTAAACGTTGGGAACTATNTTGTGGGGACCATTC
CACGGAATTCGTGANGCNCNTAACNCATTATNTTCCCGCCTGGGGAGTACGGNCGCNAGGCCTAAACTCAAANNGA
NTTNAAGGNGACCCGCNCCACCNNGCGGNCCATGCTTNTTAAATCCNATNCAACGNNGAANAACCNNTNCAATGCTTGN
CATATNCCGATNANCNGTNCANATTTNACCCCTNTTTGGNACACNNNTNTTNTGTGGATNCATAGNCATGTNNNN
AAGNTTTTTNTCNCCTAACAGNNTTTNNCTCNCNCCCNATTANNTTTTTATNCTCTTTNCCNCTCCTTTNACACAAC
TNTTCTCNCNTNTNNNTNTNTANTGTNNNGTCTCTCNCNATCNA***NNNNNNNTTNCATCNTNCTNNTC
NNTTNTACGNNAANAGNCACNNNTGNATTNTNTCTCCCNNTANCNNNCNCNCCNTCTTCTCCTTNTTTCATCCNACT
CGTNANNNTTGTGTCNCAANNATNTNTNTATNCCCTCATATTTAATTNTNTTAAACATNAAANNTNTNTAGNCGT
TNAT

Endophyte: 118

GGTGCAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCGGGCT
CAACCTGGGAACCTGCAATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGTGTAGCAGTGAAAT
GCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCTGTACTGACGCTCATGCACGAAAGCGTGCG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAGTA
ACGAAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCC
GCACAAGCGGTGGATGATGTGGTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATCCTTT
AGAGATAGAGGAGTGCTCGAAAGAGAACCCTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGCTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCCTTGCCATTAGTTGCTACGAAAGGGCACTCTAATGGGACTGCCGGTGACAAA
CCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAATGGCTGGTAC
AGAGGGTTGCCAACCGCGAGGGGGAGCTAATCCCATAAAGCCAGTCGTAGTCCGGATTTCGAGTCTGCAACTCGACT
GCGTGAAGTCGGAAT

Endophyte: 119

TTNNNCGTCTTCTGNGGGANNTGNNANTATTTGGCGTAAAGAGCTCGTANGCGGNTNGTCGCGTCTGCTGTGAAATCCC
GAGGCTCAAGTGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGG
TGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGANNTTTTT
NGGTGGGGAGCAAACAGGCTTANATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTTCCA
CGGNTTCCGTGACGCANCTAACGCATTAANTTCCCCGCTGGNGAGTACGGCCGCAAGGCTNAACTCANCAGGANTT
GACTGNNGACCCNCCCAAGCGGGTGNNCCNTNCTCGNNTAANCTNTNCCNANCANCAANAACCCCTACCACTGCTCAN
NCCATNNTACNNANNANCTTGNCTCANTANACCGGCCCGANCGNCTGNCACACTNCANANNANACNCCGNACGNTC
NAACCCGTNTCCCANTCGTNGNNGTTNNAAAAATNTNGGTCNTGCNNCCTNCACCNTNNACCCNNTNCCNANACACAA
NNCCCNNTACNNNAACCNNGACNNGTNNCNGCANGCAACAACNNNTNGCCAGNNTNCNC***NNNNNCGNNTNNNNNA
NCANNNTNCCNNCCNCCNANNAGCANNNNACTTANCTTCATANTCNCNGNCANNCCANACAAANNANNACNNACG
NAGAATNATNTCCANNCCNNATNNNCCNTNTNNNNGCTCTCNTTTNNCNAANT

Endophyte: 120

CCTCNGTCTGAGTGTGCGGATTTTGGGCGTAAAGGAGCTCGTANGCGGTTTGTGCGGTCTGCTGTGAAATCCCGAGGC
TCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTGGAA
TGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGGGTG
GGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTTCCACGGTTT
CCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGGGAGCATGCGGATTAATTCNATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAA
CGGGCCANAAGTGGTCAACTCTTTGGACACTNTAAACAGGTGTCATGGTTGTCGTCAGCTCGTGTGCGGACAA***N
NGTTGCCNNTNCATCNCNCCCCCCTNCCNCTCCANGCCCCANCATCTNCNATCNNATNCCNCCNNNATNTNCTTN
NTCATNTTCTCNCNCCNNTNCATNGNNCCNTCCNTNNNATATTCTNCCNCCNCCNCCNATCANNCNGNTTCCNATN
NNNNNCCNCCANNNNTNTNATANNNTNCCNANTNCNNAACNNNNNNNCTNTTCCNTNATCTNTCTNANNATTTCAN
CCNCCCCCNACTCCTNTNCCNCCNCCCTTTNNNTTTT

Endophyte: 121

CTNTGTCGTGAGAGTTNGTTGTTNCTGGGCNTAAAGCGCAGCTAGGCGGCTTTGTAAGTTAGAGGTGAAAGCCTGGAG
CTCAACTCCAGAATTGCCTTTAAGACTGCATCGCTTGAATCCAGGAGAGGTGAGTGGAATTCGAGTGTAGAGGTGAA
ATTCGTAGATATTCGGAAGAACACCGATGGCGAAGGCGGCTCACTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACCCCGTAAACGATGATAACTAGCTGTCCGGGGACTTGGTCTTTG
GGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGG
CTGCAAGAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATGTCCGGACGA
TTTCCAGAGATGGATTCTTCCCTTCGGGGACTGGAACACAGGTGTCATGGCTGTCGTCAGCTCGTGTGCGGAAAAA
TGTTTG***NN
NN
NN
NN

Endophyte: 122

TCNGTCGTGAGANGTTGANTCTTNTTGGGNGNNAGAGCTCGTNTGCGGATNGTNGNGTCTGCTGTGAAATCCCGAGG
CTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTANAGTGCAGTGGGAGATTGGAATTCCTGGTGTACCAGGTGG
AATGCGCAGATATCAGGAGGAACACCGATGGCNGAANGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTANATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTANTTGTGGGGACCATTTCCAC
GGTTTCTNTGACNCAGTTAACGCATTAANTTNCNCCGCTGGGGAGTACGGCCNCCNAGGCTAAAACTCAAATGAATTG
ACNNGACCCNCCCAAACGGTGGAGCATGCGNATTAATTCTATGCAANTCNAANAACNTTACCAANGCTNNNCATTCT
ACCANATCNGANNCNTAAATGGGTNNACATATTNGACACTCTNCNANNCGNNGGNGNATNGCTNGTNATNCCCTCNT
CTCNTNANGATNCCCGACNCANNCNCTNCCATATNCTCANNTCCNCTNTTCNNTCACCTNNNCCCTATATTNTNGN
CANNNGGNACANCNCTCGTTCCNNGNANTNAGCTNTCANNCNCCNNTATATAATNNNTCTCGTCNANNATTNANTTCC
ANNNTNCCNNTTACCACNACNNATNTANCNTCTANCNATTCCNNGCNCNCTNCACCTNNNATACNCNANAANAANN
ATACACNNTCANNCNTTACGNAGATNNCCNCCNCCNCTATANATNTNNATANANT

Endophyte: 123

CTCNGTCGTGACNGTTGACTCATCCTGGGCGTNNAGGGCGCGTAGGCGGACTCTTAAGTCGGGGGTGAAAGCCCAGGG
CTCAACCTGGAATTGCCTTCGATACTGAGAGTCTTGAGTTCGGAAGAGGTTGGTGGAACCTGCGAGTGTAGAGGTGAA
ATTCTAGATATTGCAAGAACACCACTGGCGAAGGCGGCCAACTGGTCCGATACTGACGCTGAGGCGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGGGTGCATGCACTTCA
GTGGCGCAGCTAACGCTTTAAGCATTCCGCCTGGGGAGTACGGTTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCTTTTGACATGTCCGGTTTGAT
CGACAGAGATGTCTTTCTTCAGTTCGGCTGGCCGGAACACAGGTGCTGCATGGCTGTCTCAGCTCGTGTCTGTGAGAAAT
ATGTTTCGNCNNTCCCCCCTTNTCCTNNNTGNNCCTTCNTACCCACCNTNNCCNNAANNCTNCNTACANACNTTNGN
NACAGNNNCCNNTNNCNCATNCCNCCNNCGANCTTCNCCNCCNCCGNTNTTNTNCCNNTGCCNCTNGNCNNNCN
CCANNTTNNCTCGCGCNTNNNNCTTTNNCTNCTCCCCCNTAATCANANCGNNANANAANTTCAANCCCCCNCNCCC
ANTNCCNCTACNNCNCATA

Endophyte: 124

CTCTGTCTGAAAGTTGNCTCATCCTGGGCGTNNAGGGCGCGTAGGCGGACTCTTAAGTCGGGGGTGAAAGCCCAGGGC
TCAAACCTGGAATTGCCTTCGATACTGAGAGTCTTGAGTTCGGAAGAGGTTGGTGGAACCTGCGAGTGTAGAGGTGAA
TTCGTAGATATTGCAAGAACACCACTGGCGAAGGCGGCCAACTGGTCCGATACTGACGCTGAGGCGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGGGTGCATGCACTTCA
TGGCGCAGCTAACGCTTTAAGCATTCCGCCTGGGGAGTACGGTTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCTTTTGACATGTCCGGTTTGAT
GACAGAGATGTCTTTCTTCAGTTCGGCTGGCCGGAACACAGGTGCTGCATGGCTGTCTCAGCTCGTGTCTGTGAGAAAT
GTTTGG***NN
CTNN
NCNN
NNNNNNCCNNTNTNTN

Endophyte: 125

TTNACTAGTTGCCTCNGTGCTGAGAGTTGCGNCATCACTGGGCGTAAAGGGCGCGTAGGCGGACTCTTAAGTCGGGGG
TGAAAGCCAGGGCTCAACCTGGAATTGCCTTCGATACTGAGAGTCTTGAGTTCGGAAGAGGTTNGGTGGAACCTGC
GAGTGTAGAGGTGAAATTCCGTAAGATATTTCGCAAGAAACACCACTGGCCGAAAGGCGGCCAACTGGTCCGATACTG
ACCCTTAAGGCCCAAANCNTGGGGAACAACAGATAATNCCTGGTANCCCCCGTAACAATNATGCCACCCGTTGGGGT
GCNTTGCCTTTNANTGGGGCCANCTTAACNCTTTTAANCCNTTTCNCCTTNGGGGNNATTCGGGGC***CCCCAAA
AANTTATAAACCCCTCAAAAANGGGANATTTTNNNGGGGGGNCNCCCNCCNNNNNNNNNGNGNGGGNNGNCC
NGGGGGGNTTAAATTTNNNNNNNNNNNNNGGACNNNAAAAAANNCCNTANCANACNNTTTTNNNNNNNNNNNNNN
NNNNNNNNNTAAGTNNNNCAANNNNNGGAGGTNNNNNNAGTNNAGTNTCCGATGGGTNNNGNNGNNNNNNNNNN
NTNTTATTTTNNNNANNGGGGNTCTTNN
NN
TNN

Endophyte: 126

CCTCNGTCTGAGAGTTGANNCTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCGG
GCTCAACCTGGGAATGCAATTTGTGACTGCATCGTGGAGTACGGCAGAGGGGGATGGAATTCCGCGTGTAGCAGTGA
AATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCCTGTACTGACGCTCATGCACGAAAGCGT
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCA
GTAACGAAGCTAACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGA
CCGCACAAGCGGTGGATGATGTGGTTAATTCGATGCAACGCGAAAAACCTTACCACCTTTGACATGTACGGAATCC
TTTAGAGATAGAGGAGTGCTCGAAAGAGAACCCTAACACAGGTGCTGCATGGCTGTCTCAGCTCGTGTCTGTGAGAAAT
GTTGAC***NCNN
NN
NN
NNNNNNANNNNNNN

Endophyte: 127

ACGGTGCAGCGTNTTGCAGATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCGGG
TCAACCTGGGAATGCAATTTGTGACTGCATCGTGGAGTACGGCAGAGGGGGATGGAATTCCGCGTGTAGCAGTGA
TGGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCCTGTACTGACGCTCATGCACGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAGT
AACGAAGCTAACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACC
CGCACAAGCGGTGGATGATGTGGTTAATTCGATGCAACGCGAAAAACCTTACCACCTTTGACATGTACGGAATCCTT
TAGAGATAGAGGAGTGCTCGAAAGAGAACCCTAACACAGGTGCTGCATGGCTGTCTCAGCTCGTGTGTGNAAAAAATN
TTGG***NN
NN
NN
NN

Endophyte: 128

AGGGTGC AAGCAGTTAATCGGATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCGG
GCTCAACCTGGGAACATGTCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGA
AATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCCTGGGCCTGTACTGACGCTCATGCACGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCA
GTAACGAAGCTAACGCGTGAAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGA
CCCCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATCC
TTTAGAGATAGAGGAGTGCTCGAAAGAGAACCCTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTTGAGAAAAANN
TTNGG***NN
NN
NN
NN

Endophyte: 129

CTCGGTGCNGAGATGTTGCGGATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGTTGTGAAATCCCCGG
GCTCAACCTGGGAACATGTCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGA
AATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCCTGGGCCTGTACTGACGCTCATGCACGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCA
GTAACGAAGCTAACGCGTGAAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGA
CCCCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATTT
GCCAGAGATGGCTTAGTGCTCGAAAGAGAGCCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGNNAAAAAA
NGTTGG***NN
NN
NN
NN

Endophyte: 130

CCTCNGTCTGAGNGTTGAGNNATTACTGGGCGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGTGAAATCCCCG
GGCTCAACCTGGGAACATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGCAGTGA
AAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCCTGGGCCTGTACTGACGCTCATGCACGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTC
AGTAACGAAGCTAACGCGTGAAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGG
ACCCGACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATC
CTTAGAGATAGAGGAGTGCTCGAAAGAGAACCCTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTNAGAA
TGTTGG***NN
NN
NN
NN

Endophyte: 131

CTCNGTCTGAGNGTTGNGNATTTTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTGCTGCTGTGAAATCCCAGGGCTC
AACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATG
CGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCGGTAAGTACGCTGAGGAGCGAAAGGGTGGG
GAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAAGTGTGTGGGGACCATTCACGCTTCC
GTGACGCGAGCTAACGCATTAAGTTCCCGCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGAC
CCGACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAAGC
GGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCG***NNAATGTT
GGNN
CNCCTTCNN
NN
TNTNTNN

Endophyte: 132

AGNNGTACCTGCTGGNCGNCGGNNNNCGATNGTTCNNGGGAANTAACAAGGAAGGCGTAAGCTTGNTTNCCTNCAG
GCCNNCTCTCAGGGTCNCGGCTGCAANNAANTACANGGCATTACTAGAGTGAGGNAGGGGAGAATGGAATTCCTGGT
GTAGCGGTGGAATGCTGCANATATCANGAGGAACACNNGATGGCGAAGGCAGTTCTCTGGGCGGTAAGTACGCTGAN
GAGCNAAGCGTGGGGAGCGAGGNGGATTAGATACNCTGGTAGTCCACNCNGNAAACGTTGNGCCNCTANATGTGGG
GACCATTCACCGGNTTCCGTGTCNCANCAACACATTAAGCNCNCCNCTGGGGAGTACGGNTTNTAAGGNTAANA
CCTCAAAANGAATCCNACNGNCGNCNCGTANAATTNNNTNCCANNATNNCCGNTTTAATTCNAATCCANTTAACGNC
AACCTTTNANNAGGNGCTTTGACCTTANANANAGAACNNNTNCNANNANNATAANGGNNNNNTCTTNTGTACNNCTCAN
TNATACTNAGNNNTGCTNNGNATNTTNTAAGCNCNCTGTCNANANAACANAANNNGNNNCTAACATCNCNCAAN
CCAANCNCNANNCNNANNTTNTNTGANAGTANACGNCNCCANNNGGNGTGNNNCTNATNACGNATCCCTGTNNNG
TCATCAATNTNACGA***NNNNGGTANTNTACNCTCANNNNACCNTGCTCCANAANCANTCGNNCNCNANNCNCCG
GTNNAANNNNNNNAACNCCNNNTTNNNCACNAATGANTNNAGANNCCGNAACACTATAAACNNNNANATCTNCTCG
NNNNNATNTNTNCCNNTNNTTNTNTANA

Endophyte: 133

GTCGACANGGNGNCCTAAGCNTGGNTTGCCGCGGTAAAGCGCACGCGAGGCGGTCTGTCAAGTCGGATGTGAAATCCCC
GGGCTCAACCTGGGAACCTGCATTGCAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGAGACTGTGGAGGAATACCGGTGGCGGAAGGCGGCCCTGGACAAAGACTGACGCTCAGGTGCGGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGG
CGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAG
AACTTCCAGAGATGGATTGGTGCCTTCGGGAACCTGTGAGACAGGTGCTGCATGGCTGTGCTCAGCTCGTGTGTGAAAA
TGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTNCGGCCGGGAACCTCAAAGGAGACTGC
CAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAA
TGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGC
AACTCGACTCCATGAAGTCGGAT

Endophyte: 134

CTCNGTGCNGAGNGTTGCGGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCGAGGC
TCAACCTCGGGCTGCAAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAAT
TGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGCATG
GGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGATGTAGGGACCTTTCACGGTTT
CTGTGTCGTAGCTAACGCATTAAGCGCCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAA
ACGGCCAGAGATGGTCGCCCCCTTGTGGTGGTGTACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCG***NNANNT
GTTGNN
NN
NN
NN

Endophyte: 136

CTCNGTCTGAGNGTTGCGNATTTTGGGCGTAAAGGAGCTCGTAGGCGGTCTGTGCGCTCTGCTGTGAAATCCCCGAGGCT
CAACCTCGGGCTGCAAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAAT
GCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGGGTGG
GGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAAGTGTGGGGTCCATTCCACGGATTC
CGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGA
CCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATACACGAGAAC
GCTGCAGAAATGTAGAACTCTTTGGACACTCGTGAACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCGNAAAAAGTT
GG***NN
NN
NN
NN

Endophyte: 137

TAGGGTGCAAGCGTNGTCCGGCATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCGAG
GGCAACCTCNGGCTTGCANTGGGTACGGGCANACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAAT
AATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGANCGAAAG
CATGGGGAGNGAACAGGATTAGATACCCTGGTANTCCNTGCCGTAANCGTTGGGCNNTAGATGTATGGACCTTTCAC
NGTTTCTGTGNCGTANNANNCNCATTANNCGCCCTNTCCTNGTNNANTACNGGCCGCTAAGGTNTAANNCCCTCANN
ANGGANTTACCTATTGCCCCCACNNTCTCGCNCCTCANGNCNATTTATTNCCGATGNCNACTCCNNTANNNCNNTNN
NTANAGGCNCCGCTTNNNNCNCNANAAAAATNTNTNTNTNTNNNNCACNCTATATTANANNCCNCTNNAACANANGTG
TGTCNANNANTNNC***NNNNNTNANCNNTCTCANNNTCATNNATNNACNTTNGNNTATAATTNNCCCTNNAACCNATN
TCTTTCNCCNTNNCTTCTATATATNNNNCCNNGNTNATANTNTNCNNNTTTANANAAAGNTCCNNTNTGTNNANNTNN
NCNNANGNNATNTTCTCNACTTNTNCNCCNNAACNCCCTCNNNCCTACATCTCNCNAGNNTNNNTNNNNATNTATA
ANATTNNCCATNTCNTANNTGTNNNCNTACNNTCCATTNGTTNTTCNTTCTCNCNCTATGNGCGNTANTATCGNTCT
CTN

Endophyte: 138

GGCAGTNGTTATCCGCNTNNTTGCNCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCGAGGCTC
AACCTCGGGCCTGCAAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATG
CGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACCTGACGCTGAGGAGCGAAAGGGTGGG
GAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAAGTGTGTTGGGGACCATTCACCGGTTTCC
GTGACGCAAGCTAACGCATTAAGTTCCCGCCTGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGAC
CCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAACG
GGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTGCTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAAGCTCATGGGATACTGCCGGGG
TCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGCTTGGGCTTACGCATGCTACAATGGCCG
GTACAAAGGCGTCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCAGTTCCGATTGAGGTTCTGCAAC

Endophyte: 139

CTCNGTNCNGAGNGTTGCGGTATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCAGAGG
CTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
ATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAGGGT
GGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGGT
TTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGG
GGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAG
AACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCGNAAAA
TGTGT***NN
NN
NN

Endophyte: 140

CTCNGTCTGAGNGTTGCGGNATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCAGAGG
TCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
TGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAGGGT
GGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGGTTT
CCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAA
CGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCGTGNAAATGT
TTGA***NN
NN
NN
NN

Endophyte: 141

CTCGTTCNGAGATGTTGCGGATTATTGGGCGTAAAGAGCTCGTANGCGGTTTGTGCGCTCTGCTGTGAAATCCCAGAGG
CTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
ATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAGGGT
GGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGA
TTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGG
GGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAG
AACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCGNAAAN
TGTGT***NN
NN
NN
NN

Endophyte: 142

TGCTCGTGTCTAGTGGGATTATTGGGCGTAAAGTAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCAG
AGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCAC
GGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGAC
CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCNATGCAACGCGAANAACCTTACCAAGGCTTGACATATAC
NANAACGGGCCATAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGCTCC***
ANAANTNTTNGNCCCCACCNTTTNNNTTNTCNCNNCNTNANTTAANNNNACNTNCTGTTNNCNCNTCNGNNNTTNC
NTNNNTNANNNATNTNTNTTTTTNGNTCNCNCNNANATCTTNCNTTCTTNCNCNTNTNNNTANANNNNNNCNTNN
TATCTNCAATCCCTCACCTNNNNANANNNNNTNATTNCNTTNTCCTNCCTNCNTCNCNTCTNGTNCNCNTNTTTC
NNCCTTTTTCCTTTATNTNGTCNACCNANT

Endophyte: 143

TAGGGTGCAAGCGTTGTCCGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCGGCTGTGAAAGCCCGGA
GCTCAACTCCGGGTCTGCAGTCGATACGGGCAGACTTGAAGTTGACAGGGGAGACTGGAATTCCTGGTGTAGCGGTGA
AATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCNCGGTCTCTGGGCAACAACGTGACGCTGAGGAGCGAAAGC
GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGCGCTAGGTGTGGGGGCCATTCCACG
GTCTCCGTGCCGAGCTAACGCATTAAGCGCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGAC
GGGGGCCCGACAAGCGGCGGAGCATGTTGCTTAATTCGATGCAACGCGAAGAACCTTACCTAGGCTTGACATGTGCG
GAAATCCTCCAGAGATGGTGGGTCCCGTAAGGGTCCGACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGAAAA
AA***NTTNGGNANNNNNNTANGCNTANATNNCNCNNATNCCGNNNGCNCNNNTNTNNATNTTTANNNATNNNATNCT
CTCCCNNTNTNCCNNNTNCTNTGNTNACATGTCTNNNAATATCCNCNTGNTNNCANTTNCNCTNNNNNTCNCNNNATN
NNGCNANNNNNNNCNCNCNCNNATATCNCNATNCNACCGCNCNNGCNCNNANNNNNNAANAACNNGCNGCAAANGNN
NNCNCNNNNANACNCCCACNNANTNNN

[illegible][illegible]

CCCTACNGTCCNGAGNNNTGCGGTATTATTGGGCGTAAAGAGCTCGTANGCGGTTNGTCGNGTCTGCTGTGAAATCCCG
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AACTTAAGTTTGNNGGGGGGNTCCCATTTNCCCACCGGGATTTCNNNTNGACGCCNAACTTAACCGCCATTTTAAANTT
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NTTGACCCGGGGGGAACCCCCNANCNANACCCAGGTGGGNAANGATAGCCNAGNATTTAAATNTNTCAATCCCNACC
NCGAAAAAACCNNTTTTCCAAGGGGTTTTTNAATNTATNTNNNAAAAAACCCGNCNCAAAAAANNNGNCCAAANTTTT
TTTGNANCNNTNTTAAAAANNNGNGNGGGGNCCTTGNTNNCNNNNNNCTCTCGNGTCNNAGAAAAANTNANCCCTACNTN
NNAATNANTATNACNNNGCGCNCCNCCGCGGATTCCCNATTTATTTCTNATNCNCTTCNATNTNNNNNGTNNCCC
CCTNTNACNNCTTTCTNNNTNTTNTAACNANCNANTAGNCTNCAATTTNTNNGCCNCCNCCNNTNNGTANAANAATAA
AANAANAANNNNCNCCNCCNANTNCCNCTATTNTATANNNNNCACNNTNACCNATNTNTANTTANC

ANTCNACCANGTNNCCNTAAGNTNTNANACCNGGGGAAGTCGTAACAAGGTAGCCGTAAGCTTGGATCCCGGGGATCCCN
TTGTATCNGCNNNNNGGTTACAATGNNAAACGNCAAACTANAGTGCAGGAAGAGGAGGACTNGGAATCCACGGNGTAC
CNGNGGGAATGCTNCATATCAGGAGGAACAACCGATGGCCAAAGGCAACATCTCTGGNCCGTANCTTACGCGTNAAGGA
GCAAAAGGGTGGGGANCNNNNGGGCTTACATACCCTGGTANTCCACCCCANAAACNNNTGGGAACCTAATTNTGGNGNN
CNTTCTCGNATTNCCNNGACCCCCCACACCAATNANCCCCNCCNAATNNNNNNANTTACNATTNNAANANTTNACTT
CTC NNTNNAATTNATNGNGACCANATANACATNNNCNGNCCNNNNNCACATNAAANNNNANCCNACCCNNAANNA
CNAANCAAGTGNNNTNANCATTTATNCANAAATNCTNCCGANCANNNNTNACTNCTTTGNNGCACTTNTNTNCCANNA
GNAGNCNANANGANCNTTANCCNCTTGTNCANAAANAATNTTNGNCTAAAGCANNCGNNACTANTCTGTNNCNC
CATNTTNTTCCNNCNNNANCTNCANANATNNGANCNCNATTTAATCNAAATCNTATCCTNANCTCAATCTNCNNGNCN
TNAANCTNACGCATNNTCCNAAANTAANANNNANANNTTNTAATCNCCNCTNTTANTNANNACCNANCCCTACCA
TANNTAACNCTTTACCANNATNANNTATANATACNANCNNNNATACTTCNATCTCCNNNGNGNNNTTACTTCTTNC
TNTNAAANNNGCCNNACAAAG

[illegible]

AGNAANTACACGGTAGNTCAGTNNGCTTGTTTNCNNNNNAGGAGCTCNTAGGCGGTTTGTGCGCTCTGCTGTGAAATC
CCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGC
GGTGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAAC TGACGCTGAGGAGCGA
AAGGTTGGGAGCAAAACAGGCTTAGATACCTGGTAGTCCACCCCGTAAACGTTGGGAACATAGTTGTGGGACCATT
CAGGTTTTCCGTGACGCGACTAACGCATTAAAGTTCCCGCTGGGGAGTAGCGCGCGCAAGGCTAAAAACTCAAAGGAAT
TGACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATA
TACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTCTG
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ACTGCCGGGCTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATATGCCCTTATGTCTTGGGCTTCACGCATGCT
ACAAATGGGCGGTACAAANGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGTTCGGATTGANGT
CTGCAACTCGACCT

GNTCNGNTCGNGNNGGTTGAGNCNGCNANCCGCGNGNNNTAGACTCGNGTCGGGANATGTTGNCNCCCCNCCCNA
ANNNTGNCCTCNTNTCGNNAANTNTACCNANTACTCGNCAGACTAGAGTGCCTGGTANGGGAGATTGGAATTCCTGGNG
TNCCGGCGGAATCGCNGCNNATATCAGGAGGAACNCCNNTATGGCNAAGGCANANCTCTGGNCCGCAANTGNCNCTNGA
NGANCNAAAGGGTGGGAGCNCNGNGNGGNCCTACATACCTGGTNGTCNCCCGCAACGCTGNGAACTATTGNGG
GNCCCTNCCACGGATTCCNGAGCCACNANNNNACGATTAACNCCCTCTCTGGGGAGTACCGCCGAGGNTAATACTAA
NTNGANTNGANGGGGACNCTCACAAACCTNTNGCCCATGCGNNTTAATTTTCATGCCANNCCGAAGAANNTTCACANC
AGCCTNTTNCCTANTCNGNACCNGCCCATACNGGCCAACTTNTATNNTACACNNGNTNNNNANTANNCGAANGCC
NNACNTCCCNCCNCGTNCCCNANNGANGCNGNANCCNCTNTCTNGTGTNTNCCNAGNNNCANCNNCACNTNTNTC
TATTNANTNATAANACGGATNNAANTTANCCNACCANCAACGNTCCATNTATNCCANCNTNCCCTCNTTCTNCN
GCANNCNACTNGNCCGTCTTATNNNNCTNNAATNNNGTNNATNCANNCNNTCCNATCAANCCGATNNTNNTATNANAG
CAANTAACNANCNAACANTNTAATCNCNCANAGNNANNNNCTGTNTNATANTT

[illegible]

AGTCNANNAGNGNANCNNNIAANNCNTGNGTNNCCN***GGGCANNGGGTAACAAGGTAGCCGTAANCTTGGTCCNG
GAAATCCCCGAGGCTCAACCTCGGGCTGCATTGGGTACTGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGG
TGTAGCGGTTGGATGCGCAGATATCAGGAGGAACCCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGTGAGG
AGCGAAGGTGGGGAGCAACAGGCTTAGATACCTTGATGTCACCCCGTAAACGTTGGGAAGTGTGGGGAC
CATTCACGGTTTCCGTGAGCAGCTAACCGCATTAAGTTCCCGCTGGGGAGTAGCGCGCAAGGCTAAAACTCAA
GGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCNATGCAACGCGAAGAACCTTACCAAGGCTTG
ACATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCACCCCTNTTCTATGTTGCCAGCACGTAATGGTGGGACCTCATG
GGATACTGCCGGGGTCAACT***NGGANGAANGTGGGGATGACNTCAAATCATCATGCCCTTATGTCTTGGGCTTCAC
CATGCTACNATGGCCGGTGACAAAGGCTGCAC TACCNNTGAAGTGGAGCGAATTCCAAAAANCCGGTCCCANNTNGGA
TGGAGGTCNGCAACTTGACCNACTGGAAAT

CTCTGTCTGACTGTTGCGTCTTTATTGGGCNTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGGCCGTGAAATCTCCACGCT
TAACGTGGAGCGTGCGGTCGATACGGGCAGACTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAAT
GCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCNNGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAACGCTG
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CCGTGCCGTAGCTAACCGCATTAAAGCGCCCCGCTGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCCACAAAGCGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTAACTGGGCTTGACATGCGCCAGAC
ATCCCCAGAGATGGGGCTTCCCTTGTGGTTGGTGTACAGGTGGTGCATGGCTTGTCTGTGAGCTCGTGTCTGAAANTGT
TNGC***NNNNNNNNCNCNCNNNNNNNNNNNNNNNNCTNNCTNCGCCNNNNNNNANNNNNNTNTNNNNCTNNTNNCT
CNNNNCNNNCACNNNNCNCNNNNNNNNCNCNCCCNCCTNCTCCNCCNNCCCCNCCTCCNNNCNATNNNNC
NNNNNNNNNNCCCCNCTNCNCNNCTTNNNNNNNNNNCNCNNNNNTNNNNNNNNNNNNCNCNNCCCCNNNNCNCN
NNNNNNNN

[illegible]

CGCTCTGTCGTGANNNGTTCNTCTTNTCTGGACATGTNGAGCTCGTAGGCGGTTTGTGCGCGTCGGCCGTGAAATCTCCA
CGCTTAACGTGGAGCGTGCGGTTCGATACGGGACGACTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTG
AAATGGCGAGATATCAGGAGGAAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGC
GTGGGAGCGAACAGAGATTAGATACCTGGTAGTCACGCTGTAAACGCTGTGGCGCTAGGTGTGGCGCATCCACGT
TGTCCGTGCGGTAGCTAACGCATTAAGCGCCCCCGCTGGGAGTAGCGGCCGAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCCTTACCTGGGCTTGACATGCGCCA
GACATCCCCAGAGATGGGGCTTCCCTTGTGGTTGGTGTACAGGTGGTGCATGGCTGTCTGTAGCTCGTGTCTGTG***NGA
ATGTTGNCNNNNCTCNNNTNCNNNNTNATTNCCNCCCTNTNTCCNCACANTCACCNNNAGANNNNNAATCANANNTNT
NANCNTNTNANNCANNNNTNCANTNTTGGTNCAATCCCTCTTNNCTTATCCTCNCCTNGCTAATCCNANATNNGNN
NNTAGNNCCNTATATNANNNNNNNNANNCNTNCNNNTNTTACNAACATNTTACATCCATNANCNNNGCTTNNNT
NCNACNTTAANTTTANGCGCCNCCNATNCCNCCNATTATTATNATC

CTCTGTCTGAGNGTTGANN***CATTTTGGGCGTAAANAGCTCGTATGCGGATNGTNGCGTCTGCTGTGAAATCCCGAGG
CTCAACCTCGGGCTGCA GTGGGTACGGGCAGACTAGAGTGC GTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
ATGCGCAGATATCAGGAGGAAACACNGATGGCGAAGGCAGATCTCTGGGCCGTAAC TGACGCTGAGGAGCGCAAAAGGG
TGGGGAGCAAAACAGGCTTAGATACCTGGTAGTCCACCCGCTAAACGCTTGGGAACTAGTTGTGGGGTCCATTCCACGG
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GGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGA
GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTC***NTCAGCTCGTGTCTNTGA
NAATGTTTGNCCCCNNTCTNNTATNCTNTTCCCNNCACAAACCTCCNANANCANNATATNNC NCCNACNACNNNNNC
TTCGNCCANNNCNACATTTACNNNCTNNATNCCCCTCCCN CN CNCGNACCNCNCTCNNNNNCCNNNNTCNCCTNTCN
NCNANTNCTNNATCNNTTNNNNCNTTACNNAAACNNGACNNTCNTANACGATNNANCANANNNTNTTNNACCNN
CCNNNNTCCNNTNTNCTCNTNTATNNNNNTANNNA TNTT

[illegible][illegible]

[illegible]

CGCTCTGTCGTGAGNTGTTGANNNTTTTGGGNANANN***GACTCGTAGGCGGNTTGTGCGGTCTGCTGTGAAATCCCGA
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GAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGCCGTAACTGACGCTGAGGAGCGAAAGG
GTGGGAGCAACACAGGCTTAGATACCTGGTAGCTACCCCGCTAAACGTTGGGAATAGTTGTGGGTCCATCCACG
GATTCGTGACGACGATAACGCATTAAGTTCCCGCCTGGGGAGTAGCGGCCGAAGGCTAAAACCTCAAAGGAATTGAC
GGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACG
AGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGAG
AATGTTGA***NNNNNCNCCCNNNNTNTCTTNNNTNNCNTNNNNNTNNANTCNNNCNCCCCCTNTCTNNNNCTCCNT
NTTTNCCNCCNNTATCNNTNNNTCNNNANNNCNNCNNNNNNCNTCNNTNCCNNNANTNNNNCNCCNCCNCCN
NNCCNCCNCCNTATNTNTNNCCNNCTTATNNCNNNCNNNNCTCTNNNTANTANATNCNCACNNCCTNANNNCNN
TCNCTCNNTCANTNCNCCANAA

CGCTCTGTCGTGANANGTTGACTNATTNTNNGGGNGN***AAGAGCTCGTAGGGCGGTTTGTCCGCTCTGCTGTGAAATCC
CGAGGCTCAACCTCGGGCCTGCA GTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCG
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CACGTTTTCCGTGACGCGACTAACGCATTAAAGTTCCCCGCTGGGGAGTAGCGCCGCAAGGCTAAAAACTCAAAGGAAT
TGACGGGGACCCGCACAAGCGGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATA
TACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTGAGCTCGTGTCTGT
GAC***NATGTTGNNNNNNNNNNNNNACCCCNCCNTNNA NNNNNCCNANCTNNNNCNANTNNNTNCANCN NATNCNTN
NTNNNTGNTCCNTTTNTGTTATNANCNTNATNNNTN NCCCTNTNCCNCCNNTTCNCNCCGNNNNCTTNNNNNTT NCCCN
ATNANNCTTCCNTNTTACNTNACNTCCNNATNTTANTNTNNAATNNACTATANCNCTCNNTCNCNCCCCTCNTTTTNNAN
TNTCCCCACCTTCCNNCTNCNCCNNCNT

[illegible]

GCTCTGTCGTGAGANGTTGACTCATNNTCGGGCGN***AANAANCTCGTACGCGGNTTGNGCGTCTGCTGTGAAATCC
CGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCG
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AGGGTGGGGAGCAACACAGGCTTAGATACCTGGTAGTCCACCCCGTAAACGGTTGGGAATAGTTGTGGGGTCCATTCC
ACGGATTCCGTGACGACGATTAACGTCATTAAAGTTCGCCCTGGGGAGTAGCGGCGCAAGGCTAAAACTCAAAGGAATT
GACGGGGACCCGACAAGCGGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATAT
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***NNGAAATGTTGNCCNTCCCTATCNCNCNTNTCCNCCNNTNATTANNCCNTCACNNNCNACNCANGNCGCTNATG
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Endophyte: 165

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GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTGG***NN
ANNANTTGGNN
NN
NN
NN

Endophyte: 166

CGCTCTGTCGTGANNAGTTGACTCNTGTCTGANNGTGTGNTCTGNTGCGGATTGTGTCGTCTGCTGTGAAATCCCGAG
GCTAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
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CTTGAAGGGANCCCAAAAAGGGGTNNGGGGGGAAGCCAAACAGGGCTAAATACCCTGGTGTCCACCCCGTAACGTTG
GAACTANTNNGGGGACCATTTCCACNGTTTTCGGNGANCCANNCTAACCNANTTAAGTTTCCCCCCCCCTTGGGGGGAA
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CCC***NN
GNNNNAATNGGNNNTNNGGNGNGGNGNNNNNNNAANAGGNNTNNNNNNNNNNCTNTCTCNCAATANTTTNNNNNN
NANTNNTNCCTCTCTNNNNNNNNNNNGGNNNNNGGNNNNNGGNGCNNNNNNNNNNNNATAAATTNNTGGGGGNNNN
NANANATNNCCNN

Endophyte: 167

GTAAGNCACAGTNGTNAATCCGGCN***TTATTGNCCGGTAAAGNGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATC
CCGAGGCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGC
GGTGAATGCGCAGATATCAGGAGGAACACCCGATGGCGAAGGCAGATCTCTGGGCCGTAACGCTGAGGAGCGA
AAGGGTGGGGAGCAAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATT
CACGGTTTCCGTGACGCAGCTAACGCATTAAGTTCCTCCGCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAAT
TGACGGGGACCCGCACAAGCGGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATA
TACGAGAACCGGGCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTG
GAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACTCATGGGG
ATACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTACGCATG
CTACAATGGCCGGGTCAAAGGGCTGCAATACCCGTGAGGGGNAGCGAATCCCAAAAAACCNGTCCAGTTCGGATTNG
AGGTCTGCAACTT

Endophyte: 168

GGNGGTGCAAGCGTTTCCGGCTTTATTGGGTTTAAAGGGTCCGTAGGCGGATCTGTAAGTCAGTGGTGAAATCTCACAG
CTTAACCTGTAAACTGCCATTGATACTGCAGGTCTTGAGTAAGGTAGAAAGTAGCTGGAATAAGTAGTGTAGCGGTGAA
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ACTAAGCGAAAAGTGATAAGTTAGCCACCTGGGGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGGGCC
GCACAAGCGGTGGATTATGTGGTTAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGGGAATTGATCGGTT
TAGAAAATAGACCTTCTCTCGGGCAATTTTCAAGGTGCTGCTGATGGTTGTCTGTCAGCTCGTGCCGTGAGGTGTTAGGTAA
GTCCTGCAACGAGCGCAACCCCTGTCACTAGTTGCCATCATTCAGTTGGGGACTCTAGTGAGACTGCCTACGCAAGTAG
AGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTACGCTTGGGCCACACACGTAATACAATGGCCGGTACAG
AGGCAGCTACACAGCGATGTGATGCAAAATCTCGAAAGCCGGTCTCAGTTCCGATTGGAGTCTGCAACTCGACTCTATG
AAGCT

Endophyte: 169

CTCGGTNCNGAGNGTTGCGGCATTATTGGGCGTAAAGGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAG
GCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGG
AATGCCAGATATCAGGAGGAACACCCGATGGCGAAGGCAGATCTCTGGGCCGTAACGCTGAGGAGCGAAAGGG
TGGGGAGCAAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGG
TTTCCGTGACGCAGCTAACGCATTAAGTTCCTCCGCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACG
GGGACCCGCACAAGCGGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGCTTGACATATACGA
GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTCTGTGAAA
ATGTTGG***NN
NN
NN
NN

[illegible]

CGCTCTGTCGTGAGANGTTGACTNTTNTNNGGGNNNNN***AGAGCTCGTANGCGGTTTGTGCGCTCTGCTGTGAAAAC
GGAGGCTCAACCTCCAGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCTGGTGTAGCG
GTGGAATGCGCAGATATCAGGAGGAACACCGTAGTGGCGAAGGCAGATCTCTGGGCCGTAAC TGACGCTGAGGAGCGAA
AGGGTGGGAGCAAAACAGGCTTAGATACCTGGTAGTCCACCCGTAACCGTTGGGAACATAGTTGTGGGGACCATCC
ACGGTTTCCGTGACGCAGCTAACCGATTAAAGTTCCCCGCTGGGGAGTAGCGCCGCAAGGCTAAAACCTCAAAGGAATT
GACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCCTTACCAAGGCTTGACATAT
ACGGGAACGCTGCAGAAATGTAGAACTCTTTGGACACTCGTATACAGGTGGTGCATGGTTGTGCTCAGCTCGTGTCTGTG
***NGNANGTTGNNNCNCTTTNTCNCNNNNNGCCTTTTANNNCTCANNNTTTTTCCNNNNNTNNCCTCCNCCNANNNNT
ACNNACNNNNNNCCNCTCTNTNATNNTTTTNCANNNANNNCCNNTNANTTNACNNCCCCCNCCNCCNTTTNTNCC
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CNNNTNNNT

[illegible][illegible][illegible]

Endophyte: 175

TAGGGTCCGAGCGTTGTCCGGATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTGCGGTTGTTTCGTGAAAACTCACAG
CTTAACCTGTGGGCTGCGGGCGATACGGGCAGACTGGAGTACTGCAGGGGAGACTGGAATTCCTGGTGTAGCCGGTGG
AATGCCGAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTAACGTGACGCTGAGGAGCGAAAGCN
GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACCGGTGGTACTAGGTGTGGGTTTCCTTCCTTGG
GATCCGTGCCGTAGCTAACGCATTAAGTACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCNATGCAACGCGAAGAACCTTACCTGGGTTTGACATGCACAG
GACGCCGCGAGAGATGTGCGTTCCCTTGTGGCTGTGTGCANGTGGTGCATGGCTGTCGTCACTCTG***NNNGAAAA
NNNNNNNGNN
NN
NNNNNNNNNAGNN
NTNNNNNNNTNN

Endophyte: 176

TACGGNCCAGNGTATGCGGATTATTGGGCGTAAAGAGCTCGTANGCGGTTNGTTCGCGTCTGCTGTGAAATCCCGAGG
CTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTATGGGGAGATTGGAATTCCTGGTGTAGCCGGTGG
ATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAGGGT
GGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGA
TTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACG
GGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAG
AACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCACTCGTG***NGNNGAN
NNNGTTGNN
NN
NN
NNNNNNNN

Endophyte: 177

NTCGNGCAGNGNN***ATCGCGGATTATTGGGCGTAAAGAGCTCGTNTGCGGNTNGNCGNGTCTGCTGTGAAATCCCG
AGGCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTATGGGGAGATTGGAATTCCTGGTGTAGCCGGT
GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCAC
GGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAC
GAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCACTCGTG***N
NAAAATGTTGNN
NN
NN
NN

Endophyte: 178

CTACGGTCTNACATGTTGCGGCATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTGCGGTTGTTTCGTGAAAACTCAC
CAACCTCGGGCTGCAGTGGGTACGGGCAGACTANAGTGCNGTANGGGAGAATTTGGNAATTCNNNNNNNNNNNNNNNN
AGNNAANCTGAAGGATTCNAGAANGANCNNGGGAAGCCATCTCNCCNNANTNACCTNNGGACNAAAGGNGGGNAC
CNACCNGCTTNGATCCCTGGTNTTCNCCCGTAACCTTGAANTNAAATNNGGGGTCNTTCCCGGTTTCGGAANCNTN
AANCTTTAGNTTTCNCCCGGATCNGCCCAAGGTTTACTTCNAAANGNNTTNNCGGNGCCCCACCCTACNGCGGACN
NTNCTNNTTANNTTTNTTNTNCCAAAACCTTNCNNGNNTTNCNNTTNTCNAAAAANNCCNAAACNTNTGCCNCN
TTTCNNNCNNANCAANANAGGGNCGNNNNCTTNTCTCCANNTTNCNNTCNCNACAAACNTNTCCNCCCTCCCNCC
TCNNAAAATATNACNNTNNNNNCNCCCTCCCNCAACNNNNANCTATTNCTTACNTNTNCCCTNCCCTCCNNTTN
TTT

Endophyte: 179

CTCTGTCTGAGANTTGCGGATTATTGGGCGTAAAGGACTCGTGTGNGGATANGTTGGTCTGCTGTGAAATCCCGAGGCT
CAACCTCGGGCTGCAGTGGGTACGGGCAGACTANAGTGCNGTANGGGAGAATTTGGNAATTCNNNNNNNNNNNNNNNN
AGNNAANCTGAAGGATTCNAGAANGANCNNGGGAAGCCATCTCNCCNNANTNACCTNNGGACNAAAGGNGGGNAC
CNACCNGCTTNGATCCCTGGTNTTCNCCCGTAACCTTGAANTNAAATNNGGGGTCNTTCCCGGTTTCGGAANCNTN
AANCTTTAGNTTTCNCCCGGATCNGCCCAAGGTTTACTTCNAAANGNNTTNNCGGNGCCCCACCCTACNGCGGACN
NTNCTNNTTANNTTTNTTNTNCCAAAACCTTNCNNGNNTTNCNNTTNTCNAAAAANNCCNAAACNTNTGCCNCN
TTTCNNNCNNANCAANANAGGGNCGNNNNCTTNTCTCCANNTTNCNNTCNCNACAAACNTNTCCNCCCTCCCNCC
TCNNAAAATATNACNNTNNNNNCNCCCTCCCNCAACNNNNANCTATTNCTTACNTNTNCCCTNCCCTCCNNTTN
TTT

GGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGGTCTGCTGTGAAATCCCGAGGCT
CAACCTCGGGCCTGCAGATTGGGTACGGGCAGACTAGATGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAAT
GGCGAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGCAAAAGGGTGG
GGAGCAACACGGCTTAGATACCTTGGTAGTCCACCCGTAAACGTTGGGAAGCTAGTTGTGGGGACCATTCACAGGTTTC
CGTGACGCAGCTAACGCATTAAGTTCCTCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGA
CCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAAC
GGGCGCAAAATGGTCAACTCTTTGGACACTCGTAAACAGGTTGTCATGGTTGTGCTGAGCTCGTGTCTGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCTCGTTCTATGTTGCCAGCAGCTAATGGTGGGAAGCTACGGGATCTGCCGGG
GTCAACTCGGAGGAAGTTGGGGATGACGTCAAATCATCATATGCCCTTATGTCTTGGGCTTACGCATGCTACAATGGCC
GGTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCCAAAAGCCGGTCCCGATTGCGGATTGANGTCTGCAACT

[illegible][illegible]

CGCTCTTGTGCTGANNNGTTGACTCATTGTNGNGNANNNNGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCC
GAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGG
TGAATGCNGCCAGATATCANGGCAGGAACCACCCGAATGGCCGAAGGCAGATCTCTGGGCCGTACTGACGCTGAGG
AGCGAAAGGGTGGGGAGCAAACAGGCTTAAATCCCTGGTAGTCCACCCGTAAACCGTTGGGAACTATTGTGGGGGTC
CATTTCCCACGGATTTCNTNGGACNNCANCTTAAACNCAATTAANANTCCCCNTNNCTTGGGGNGNANTNNCN
NGCNCNNTANGGNGNNTTAAAAATTAAATGGNNTTTTCTCCGGGNGNCCCCCTNNNNNCTNCNNGNCCCCCCC
CCNNCCTTNNNNNANTNANGANCTANCCNCCNNNCNTAACCTTANNGNNCANGNTTNGNTAGAGNAANCNNAACCGN
NCCCCGTANANATGNCGTCCNCCTCNGNGGGAACCNNGTCTTAANCCAAGNGNNNCNCTAANNNTCCNCCCANANTC
AANTNTCTNACATACNTTTCNCNCNCTNCCNTCTCCCNCCCGNCCNNTTNTCNCTCENNCCATNCNCCTNNCNTCATC
GGGNCNGNCCACATCNTATCATCNNTCNATANNNCNTCTCTCTNCNTNCTATCTNNCACTCTCCCTNTCNACNTACN
NTNNNCANCNNTNNCNNTNCACATATGACGTNNNNNCTCCNCGTCNACCNNNCTCGNNGNATNNATNNNTNGNC
NCNCGTCTCTTTTNTCNTTCCCNNTACNCATNATTTTCGNT***N

[illegible]

Endophyte: 185

ANCTCGTGNCTGNTGNAGTTNTTGGGNTN***ATTACTCGGGCAGTAAAGCGTGCGCAGGCGGTGATGTAAGACAGATGT
GAAATCCCCGGGCTCAACCTGGGAACGCATTTGTGACTGCATCGCTGGAGTACGGCAGAGGGGGATGGAATTCGCG
TGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCNAGTGGCGAAGGCAATCCCCCTGGGCTGTACTGACGCTCAT
GCACGAAAGCGTGGGGAGCAAAACAGGATTAGATACCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGT
CTTCACTGACTCAGTAACGAAGCTAACGCGTGAAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGG
AATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGTTTAATTTCGATGCAACGCGAAAAACCTTACCCACCTTTGAC
ATGTACGGAATCCCTTAGAGATAGAGGAGTGCTCGAAANAAAAACCGTAACACAGGTGCTGCATGGTTGTCNTCAGCTC
GTGTGGGAAAAATCGTT***NANANNCNNAANNNNNNNNATNTNTNTTNNNNNTTNTATTNNNCATACANCTANNNNTNACN
TNNATTNTAACTNCTAATCCNATTNTNNCANNNNNNNCTCTNNNNNNNTNTNTTTCNNCTNNTNTTCCACNNGNTCCC
NNNNNNNNNCCANNNTCCNNNACNCTNTCTNTNNNNNTTNTNTTNTTTCNNNCCNTNNTTNNNNNTTCCCCCTAN
NNNNNTGCCTANNTCTCTTTCCATCCNCTNT

Endophyte: 186

N***TACGGNGCAGNGTNTCGCGGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCG
AGGCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCNAGTGGCGAAGGCAGATCTCTGGGCCGTAACGACGCTGAGGAGCGGAA
GGGTGGGGAGCAAACAGGCTTAGATACCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCA
CGGATTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTG
ACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATA
CGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGGAA
AA***NNTNTTGNCCNN
NN
NN
NN

Endophyte: 187

CCTCTGTCTGAAAGTTGGCTATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCGAGGC
TCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCGGTGGAA
TGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGACGCTGAGGAGCGGAAAGGGTG
GGGAGCAAACAGGCTTAGATACCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGGTTT
CCGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAA
CGGGCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTG***NANATG
TTGNCNNNNNCNATNCTNNNNCTNTNNNTANNCACTCTATCTNTNTNTANTCACTANNNNNNATCNNNATNTCNTA
AAATNNNTNATTTNACAANNCANANGANNANAACNCTNACNTNNTNAAANNCCCTTNTNNNTNAAANGNNCNTCNNANAA
NGNNATNNGNNCCCNANTCNNNTNCCCTNGNCCNACCAACNCAANNNGNTNNNNNNNCCNNCTNTANANTNNCTT
CCCCNCTNTNNNNNTNNNNTTTNTNN

Endophyte: 188

CGTNGGTNCNGNGTNTGCGGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCGAG
GCTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCAGGTG
GAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGNNACTGACGCTGAGGAGCGGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCTGGTAGTCCACCCCGTAAACGTTGNGAACTAGTTGTGGGGACCATTCCAC
NGTTTCCGTGACGCANCTAACGCATTAANTTCCCNCTGGGGANTNCGCCGCANGGCTAAACTCAAAGGAATTGA
CGNGACCCCCACAAGCGGNGGANCATGCGGATTAATTTCGATGCAACNCGAANAACCTTACCAAGGCTTGACATATAC
NAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCNTAAACANGTGGTGCATGGNTGTCGNCANCTCGTGTNGGNA
AANATTTG***NN
NN
NN
NN
NATNTNNNNN

Endophyte: 189

CTCNGTCTGAGAGTTGCGGATTATTGGGCGTAAAGAGCTCGTANGCGGTTNGTCGCGTCTGCTGTGAAATCCCCGAGGCT
CAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGAGATTGGAATTCCTGGTGTAGCAGGTGGAAT
GCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGACGCTGAGGAGCGGAAAGGGTGG
GGAGCAAACAGGCTTAGATACCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTC
CGTGACGCAGCTAACGCATTAAGTTCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGA
CCCGCACAAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAAC
GGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGGAGAAATGTT
GCC***NNNNNNNCCNCCNN
NCTCNCNNTCNCNNTTNCNNTANCNCCNN
TNNNTNNNNNTTNN
CTNTCNCNNNNATNCNTCNCNNNTN

Endophyte: 190

GTCNAACAAGGTAGCTCGTAAGCTTGGTTCCCGGGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCG
AGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCAC
GGATTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATACAC
GAGAACGCTGCAGAAATGTAGAACTCTTTGGACACTCGTGAAACAGGTGGTGGCATGGTTGTCTGCTCAGCTCGTGTCTGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACTCATGGGATACT
GCCGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTACA
ATGGCCGGTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCGATTTCGGATTGAGGTCTG
CAACTCGACCTCATG

Endophyte: 191

CCTCGGTGCTNANNGTTGCGGATTACTGGGCGTAAAGAGTTCGTAGGCGGTTTGTACGTCGCTGTGAAAACCCACCG
CTCTCAACCTGGGCGCTGCAGGCGATACGGGCAGACTTGTAGTACTGCAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAA
ATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTAACGTGACGCTGAGGAACGAAAGCGT
GGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCGCTAGGTGTGGGTTCTTCCACGGGA
TCTGTGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCNATGCAACGCGAAGAANCCTTACCTGGGTTTGACATATACTGG
AAAGCTGCANAGATGTATCCCCNTTGTNGNCNCATACAGGTNGNGCATGGCNGTGTNNCACCTCTNGTNTGNNANT
NNTT***NNNNNNNCCCTTTGTCCNCNCNCCNCCNNTGCGCNCNGNTTANNTAANNNTNTTCCNNTNCTNNTNATNT
GNNCTNCCANNTATNANCTNCCNNTNNTCNAATCCTCCNTTNNCGNANACCCCNANTNNTTCTTNNNNNTNTCCG
NAAATNTAANNNNNNANNGNNNCACTNNNCNNTNANACCNCNANNGGTTNTNTNCCCCCCTNANNNCNCTANNTT
TNTC

Endophyte: 192

CTCGTGTCTGAGATGTTGCGNCATCACTGGGCGTAAAGGGCGCGTAGGCGGACTCTTAAGTCGGGGGTGAAAGCCCA
GGGCTCAACCTGGAATTGCCTTCGATACTGAGAGTCTTGAGTTCGGAAGAGGTTGGTGGAACGTGCGAGTGTAGAGTGA
GAAATTCGTAGATATTCGCAAGAACACCAAGTGGCGAAGGCGGCCAACTGGTCCGATACTGACGCTGAGGCGCGAAAGCGT
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGGGTGCATGCACT
TCAGTGGCGCAGCTAACGCTTTAAGCATTCGCGCTGGGGAGTACGGTGCGAAGATTAAAACCTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGCAGAACCCTACCAGCTTTTGACATGTCCGGTTT
GATCGACAGAGATGTCTTTCTTCAGTTCGGCTGGCCGGAACACAGGTGCTGCATGGCTGCTCGTCAGCTCGTGGGAAAA
AAANNTNGG***NN
NN
NN
NN

Endophyte: 193

CTCGTGTCTGAGNGTTGCGGCATCACTGGGCGTAAAGGGCGCGTAGGCGGACTCTTAAGTCGGGGGTGAAAGCCCAAGG
GCTCAACCTGGAATTGCCTTCGATACTGAGAGTCTTGAGTTCGGAAGAGGTTGGTGGAACGTGCGAGTGTAGAGTGA
AATTCGTAGATATTCGCAAGAACACCAAGTGGCGAAGGCGGCCAACTGGTCCGATACTGACGCTGAGGCGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGCCGTTGGGGTGCATGCACTTC
AGTGGCGCAGCTAACGCTTTAAGCATTCGCGCTGGGGAGTACGGTGCGAAGATTAAAACCTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGCAGAACCCTACCAGCTTTTGACATGTCCGGTTTGA
TCGACAGAGATGTCTTTCTTCAGTTCGGCTGGCCGGAACACAGGTGCTGCATGGCTGCTCGTCAGCTCGTGTGGNAAAA
NTTTGG***NN
NN
NN
NN

Endophyte: 196

CGCTCNGTCGTGAGANGTTGCNTNTTNGGGAANNTN***GACTCGTANGCGGNTTGTGCGCTCTGCTGTGAAATCCCG
AGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCCGATGGCGAAGGCAGATCTCTGGGCCGTAACGTGACGCTGAGGAGCGGAAA
GGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTC
ACGGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATT
GACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCCTACCAAGGCTTGACATAT
ACGAGAACGGGCCANAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCTGTCAGCTCGTGTGCG**
*NGAAATNTTGNNTANATNTNTNTTCCCTTNNNNNGNCCNCCNNTATNNNTTNTNTNANNNCNNTATCC
CANITCTAGNTTCTNANNTNTCCNTNTTATTTTCTTTTNTNATTNCCNATTGTNNCTNCTTTTCCNTNNNNNN
CCNTANNTNATTTNCNCCNNTTATNNNTTNTNNTNCTATNTTNNNCTCNANNANACNCCNATNTATNTANNA
TCACATCNATACNCCCGCCNAANANTNGC

CGCTCNGTCGTGAAANGTTGNNNTNTNTNGGGGAANNNTNGAGCTCGTANGCGGNTNGTCGCGTCTGCTGTGAAATCCCGA
GGCTCAACCTCGGGCCTGCAGTGGGTACGGGACAGCTAGAGTGGCGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTG
GAATGCGCAGATATCAGGAGGAACACCNGATGGCGAAGGCAGATCTCTGGGCCGTAAC TGACGCTGAGGAGCGGAAAG
GGTGGGGAGCAAAACAGGCTTAGATACCTGGTAGTCCACCCCGTAACGTTGGGAACATAGTTGTGGGGACCATCCAC
GGTTTCGTGACGACANTAACGTCATTAAGTTCCCCGCTGGGGAGTAGACGCGCGCAAGGCTAAAACTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGCGGANCATGCGGATTNATTCNATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAC
CATAACGGGGCCACAAATGGNCAACTCTTTGGACACTCGTCAACANGNGGTGCATGGNTGTCGNTCANCTNGTGNCNTN
AACATGTTNGACGCTNTCNACCNCNNNCCTTNCCTTTCN***NNNCGNNNNCCTCCNNNGNNAANNNTATNCTTTNTA
ATAGCTNCCGNCNTTAACCCNTTNTTNNNGATCNCGNNTNNAATNCCNNNTNAGNCTNTTNNCNATANTTNNCCAN
ANTNCCNCNTANTNNANNCGTCTNTNTNNNACC***CNATNTNATNTNATNTNTNTTTCNCCNCNTCNTTAT
TGCANGCNNCTTNGNTNTANNCCNCCNNTANTCNCNNNANNNTATCAAT

CGCTCNGTCGTGAGANGTTGCNTNTNTNTNGGGNANNTN***GAGCTCGTAGGGCGGTTTGTGCGCTGCTGCTGTGAAATCCC
GAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGGGTAGGGGAGATTGGAATTCCTGGTGTAGCGG
TGGAAATCGCGAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCGTNACTGACGCTGAGGAGCGGAAA
GGGTGGGGAGCAAAACAGGCTAGATAACCTGGTAGTCCACCCCGTAAACGTTGGGAAGTATGTTGGGGACCATCCA
CGGTTTTCCGTGACGCGAGCTAACGCATTAAAGTCTCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTG
ACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTGATGCAACGCGAAGAACCCTTACCAAGGCTTGACATATA
CGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTGCTGCTAGCTCGTGTGCT**
*NAANAATGNTTGTNCCCCCTTTCCNCCNCNCTCTTTNTNNNCATATNATNNNCATTAACNCTNNNNCTNNNNNT
CNNNTATTNCTNTNTNCCNCTANNNTNCCNCCCCNNNCNANNNNTATTTCTNTCTNTANCNCNCCNANCCCCNTATNTN
TNTNCTNCTNNNTNCGCNCNNNTNTANNNTNTNNNTTCCTTATCNCCTNCCCNNTTTTNCNCTATCTNTNTNTTTC
TCCCCCCCCCTNGACNNCTNATNNNNCTCNCNT

CNNCTGTCGCGTAAANGTTGN***CTCGTGTCGNGAAANTTGA CTGATGCGGATTGTCGCGTCTGCTGTGAAATCCCG
 AGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC GGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
 GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCGGTAAC TGACGCTGAGGAGCGAAAG
 GGTGGGAGCAAAACAGGCTTAGATACCTTGGTAGTCCACCCCGTAAACGTTGGGAAC TAGTTGTGGGACCATCCAC
 GTTTCCGTGACGACGAGCTAACCGATTAAAGTTCCCGCCTGGGAGTAGCGGCCGAAGGCTAAAACCTCAAAGGAATTGA
 CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCNATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAC
 GANAACGGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGCGGTGCATGGTTGTC***NTCAGCTCGTGTNGT
 GAGCATGTTGCGTTNTNCTNCNTNANNCANTNCATTNATNCACNCGNCNCTCNNTTNCNCNTTGTNTNTNNTNCNCCCN
 TNTTCCACNNATNCCCGNTTNTCCCNCCCCCTTNNNNNNCNCCTNNCNCTNTTNTCTCNCCTCCNCCCCCTCCCCC
 CTANATNTGATCCTNGATCTACNNCNCNTTTACTANNANTTCTTTTNNANCCNATNNAATNNNCCTANNCTNNAAT
 NGTTNCCCTCATNCCCCNCNCNTTNCNTNT

[illegible]

CGCTCTGCTGAGTGTGCGGTATTATTGGGCGTAAAGNAGCTCGTANGCGGTTNGTNGCGTCTGCTGTGAAATCCC
GAGGCTCAACCTNGGGCTGCATTGGGTACTGGGCAGACTAGAGTGCGGGTAGGGGAGATTGGAATTCCTGGTGTAGC
GGTGAATGCGCAGATATCAGGAGGAACACCAGATGGCGAAGGCAAATCTCTGGGCCGTNACTGACGCTGAGGAGCG
AAAGGGTGGGAGCAAACAGGCTTAGATACCCTGGTAGCTCCACCCGTAACAGCTTGGGAAGTGTGGGACCAATT
CCACNGTTTCCGTGACNCATTAACGCGATTAAAGTTCCCNCTGGGGAGTAGCGGCCGANGGCTAAAACTCAAAGGAA
TTGACGGGGACCCGCACAAGCGCGGAGCATGCGGATTAATTCTGCAACGCGAANAACCTTACCAAGGCTTGACNT
ATACNANAACGGGCCAAAAATGGNCAACTNTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGT
GGAAATGTTGNC***NNNNNNNNNNNTNANANNNNNNTNNTNNNNNNNNNNNNNNNNNTCNNNNNCNNNNNNNNNN
NNNNNNCNTTNNNNNCNTNTNTNTNTNTNNCNNNNCNCNNNTNNNNNNNCCNNCNNNNNTNTNNNNNCNCCNTNNNCNTT
NNCNCNNNTNNNNNTNNCNCNNNNNNNNNTNTNNNNNNNNNNNNNNCNCNNNTNNNNNNNCCNCCNCCNTCCNN
TNNCCNCCNNNNCCNNATNTNNCN

Endophyte: 202

CGCTCNGTCGTGAGAAGTTGNN***TCGTNTCGNGAANNNTTGA CTCTGTANGCGGATTGTGCGCTCTGCTGTGAAATCCCCG
AGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC GGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCAC
GGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGCGCCGAAGGCTAAACTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAC
GAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGA
GAATGTTGTT***NCCNANCNNACNCCNNCNTTTCCNCCCCCNNTTNNNNNTCNTTTTNNNNNNNNNNCTNNNNCCC
NCNNNNNCCTTTNTNNNCNNNNNCNNNTCTNNTNCATANNNCNCCNNNTCNNNNANNNCCCTTCCNTCNACNCNATC
TNTNNNTNTACTNNGTNTCCCCCTTNTNANTNCCNNTTTTNNNNNNNCNCACCCNNNNCTTNNNTNCTNTNTNATNT
TATNTCCCCCNACNTTNNCNCNANTCNTTNNNTT

Endophyte: 203

GTCNACAAGGTAGCCGTAAGCTTGGTTCCCGGGTAANGCAGCTCGTAGGCGGTTTGTGCTGTCTGCTGTGAAATCCCCG
AGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC GGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCAC
GGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGCGCCGAAGGCTAAACTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAC
GAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGA
GATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACTCATGGGATAC
TGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGTCTTGGGCTTCACGCATGCTAC
AATGGCCGGTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGTTCGGATTGAGGTCT
GCAACT

Endophyte: 204

CGCTCNGTCGTGAGATGTTGCGTATTATTGGGCGTANAGGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCG
AGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC GGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
GGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAG
GGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCAC
GGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGCGCCGAAGGCTAAACTCAAAGGAATTGA
CGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAC
GAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGA
AAATGTTG***NN
NN
NN
NN
NN

Endophyte: 205

CGCTCNGTCGTGAGAGTTGCGGTATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCCG
GAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC GGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
GAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAG
GTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACG
GTTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGCGCCGAAGGCTAAACTCAAAGGAATTGAC
GGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACG
AGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGAAA
ATGTTGC***NN
NN
NNNNNTNN
NN

Endophyte: 206

AGTCNAACAGGTAGCCGTAAGCTTGGTTCCCGGGTAAAGCAGCTCAAGGGCGGCTTGTCTNTGTNTGCTGAAATCCCC
GAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC GGTAGGGGAGATTGGAATTCCTGGTGTAGCGGT
TGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAA
GGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCA
CGGTTTCCGTGACGCAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGCGCCGAAGGCTAAACTCAAAGGAATTG
ACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATA
CGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGA
GATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACTCATGGGATAC
TGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGTCTTGGGCTTCACGCATGCTAC
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CGCTCTTGTCGTGANNNGNTTGGACTCGTGTCTGTGAAAAGTTGNCCTCGTNTGCGGATNGTNGCGTCTGCTGTGAAATCCC
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GACGGGGACCCGCACAAGCGGGCGGAGCATGCGNNATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACAT
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ACACTCTNCCNNGTTTCAACNCCNNNNTCTCACNNCTTCTNTNTNCCNCCCNCCCTCNANNTNNTTNTTNC
TGTCNNNGTCCANNANCNGNTATTNTTANCGATTNTNCNTATTCCTNACNTTANNCTGTACNANTANTNTTATT
ATNCCNCCANNTNANCCCCNCCNCGNCNCAATACNCTTNTTTT

[illegible]

TTGGGCGTAAGAGCTCGTAGGGCGGTTTGTGCGGTCTGCTGTGAAATCCCGAGGCTCAACCTCGGGCCTGCAGTGGGTAC
GGCAGACTAGAGTGCGGTAGGGGAGATTGGAATCTCTGGTGTAGCGGTGGAATGCCAGATATCAGGAGGAACACC
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CGGATTAATTTCGATGCAACGCGAAGAACCCTTACCAAGGCTTGACATATACGAGAACGGGCCAGAAATGGTCAACTCTT
TGGACACTCGTAAACAGGTGGTGCATGGTTGTCTCAGCTCGTGTCTGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGC
AACCCCTCGTTCTATGTTGCAGCAGCTAATGGTGGGAACTCATGGGACTGCCGGGGTCAACTCGGAGGAAGGTGGG
GTGACGCTCAAATCATATGCCCTTATGTTTGGGCTTCACGCATGCTACAATGCCCGGTACAAGAGGCTGCAATACC
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[illegible]

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CGCAGATATCAGGAGGAACACCGATGCGGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAGGGTGGG
GAGCAAAACAGGCTTAGATACCTCGTAGTCCACCCCGTAAACCTGTGGGAACTAGTTGTGGGGACCAATCCACGGTTTC
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GGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTGAGCTCGTGTGCTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCTCGTTCTATGTTGCCAGCACGTAAGTGGTGGGAACCTATGGGATACTGCCGGGG
TCAACTCGGAGGAAGGTGGGATGACGTCAAACTCATCATGCCCTTATGTCTTGGGCTTACGCATGCTACAATGGCCG
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[illegible]

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GCGTGGGGAGCAACAGGATTAGATACCCCTGGTAGTCCACGCTGTAAACGATGTCGATTGGAGGTTGTGCCCTTTGAG
CGGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCTGGGGAGTAGACGCGCGCAAGGTTAAAACTCAAATGAATTGAC
GGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAATTGATGCAACGCGAAGAACCCTTACCTGGTCTTGACATCCACA
GAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCTGTCAGCTCGTGTGTGAA
ATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTATCCTTTGTTGCCAGCGGTTNGGCCGGGAACCTCAAAGGAGACTG
CCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACA
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CAACTC

CGNTCNTGNTCNGGANN**GTTTGGACTCGTTGTCGTGAAAGTTGAGCTCGTATGCGGATNGTNGCGTCTGCTGTGAA
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AGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGTGCAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAG
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ATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCCGTCANCTCGTG
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NCTACNCCNANNNCNANNCAGCATTCANNCTTCNTNCTTTANCCCNCCCTCACAATTAACNNCNNNCACTTCCNT
CCNCAANNNTGNNCENNNTGCTCENNNNNTCNCNNTNCTACNCCNNGTNGCCNNNCTATCNCNNAATTNCNNTCC
CTNCCNNGNTCTCNCNCNGTCTCNCNCTCENNANTTT

[illegible]

GAGGTGCAAGCGTTAATCGGATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
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GGCTTCCGGAGCTAACCGCTTAAATCGACCGCTGGGGAGTAGCGGCCGAAGGTTAAACACTCAATGAATTGACGGGG
GCCCCACAAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCCTTACCTGGTCTTGACATCCACAGAACT
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TGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGG
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TCGACTNCATGAAGTCNGAATCGCTA

Endophyte: 217

GTACNAACAAGGTAGCCGTAAGCTTGGTATCCCGGGTAAAGCAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCC
CGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCG
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AGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTC
ACGGTTTCCGTGACGAGCTAACGCATTAAGTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATT
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GGATACTGCCGNGGTCAACCTCGGANGAAGGTGGGGATGACNTCAAATCATCATCCCTTATGTCTTGGGCTTTACGC
ATGTTNNAATGGNCNGGTNCAAANGGCTGCATTANCGTGAGGNGGAGCNNAATTCCTCAAAAAACCCGNTCCCATTT
CGGATTNNANGTCTNAANCTCCANCTNATN

Endophyte: 218

CTCGTNNAGNGTTGCGGATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTNNNGTCTGCTGTGAAATCCCGAGGCT
CTCAACCTCGGGCTGCANTGGGTACTGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAAT
GCGCAGATATCAGGAGGAACACCAGATGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGAGGAGCGAAAGGGTG
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ACGGGCCANAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTGCATGGTTGTCGTACGCTCGTGTCTNNAAAA
NTTTGG***NN
NN
NN
NN

Endophyte: 219

TANGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCACG
GCTCAACCTCGGGCTGAGGGTCAATTGGAAGTGGGGAACCTGAGTGCAGAAGAGAAAAGCGGAATTCACGCTGTAGCGGTG
AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTGGTCTGTAAGTACGCTGAGGCGCGAAAGCG
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CAACTCTAGAGATAGAGCGTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTACGCTCGTGTCTGTAG
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CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAA
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AACTCGCCTACAT***N

Endophyte: 220

TCTCNGTCNTGAANNNTGGCTNTTATTGGGNNTAAAGAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAGG
CTCAACCTCGGGCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTGGGGAGATTGGAATTCCTGGTGTAGCGGTGGA
ATGCGCAGATATCAGGAGGAACACCAGTGGCGAAGGCAGATCTCTGGGCCGTNACTGACGCTGANGANCAGAAAGGGT
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NNCTCTCNTNACNTNAANTTTCNCTNNCNTACT***NTTTTTCNCCCGTNTCNGCTTNNNCNCCNTNAATCATCN
GNNNNNTANNGCTGCTCNANATNTNCANNTTTTNTCTATTTCTNNTCTACNNNCANNTNNTNCTGACCCNNTN
NTCCNTTCTGTCTNNNTNNTNCGCTNNTNTNCAATTTNTATNTCNCCNATANNANNTTNNNTNATAANGTATANT
NTNATAANNANATTANANNANNTATTNTNTNACCCCNNTTANCCNCCATANTATNNAT

Endophyte: 221

CGAGGGTGCAAGCGTTAATGCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGCTGTCAAGTCCGATGTGAAATCCC
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TGAAATGCGTAGAGATCTGGAGGAATACCGTGGCGAAGGCNNGGCCCTGGACAAAGACTGACGCTCAGGTGCGAA
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GGCGTGGCTTCCGAGCTAACGCGTTAAGTCGACCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGA
CGGGGGCCGCACAAGCGGTGGAGCATGTGGTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAG
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AAA***NNNTNGGNNN
NN
NN
NN

Endophyte: 222

NGAGGTGCAAGCGTTAATGCGGATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
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GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGA
ACTTNCCAGAGATGNNTTGGTGCCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTACGCTCGTGTGG***NNA
AANAANNTGGGNN
NN
NN
NN

Endophyte: 223

NNAGGTGCAAGCNTTN***ATGCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCC
CCGGGCTCAAGTGGGAACTGCATTGCAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTG
GTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACAAAGACTGACGCTCAGGTGCGCA
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GAGAACTTNCCAGAGATGNNTTGGTGCCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTACGCTCGTTGAAA
AAAA***NTTNGGNN
NN
NN
NN

Endophyte: 224

CTCNGTCTGACATNTTNGGAATTCTGGGCGTAAAGCGNCGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTC
AACCTGGGAATGCATTGCAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATG
CGTANAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACAAAANNCTTGCNCTTCANGTTGCCAAANCG
TGGGAGCAAACAGATTAAATCCCCTGGNNNTCNCNCNTAAACCAANTNTNNACCTTNGAAGGTTTGNCCCCCTTNA
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NNNNNNNNNNNGNCNTCNNCCNCCNNG

Endophyte: 225

CTCGGTNCNGAGTGNTGCGGTATTATTGGGCGTAAAGAGCTCGTGTGCGGATATGNTGGTCTGCTGTGAAATCCCCGAGG
CTCAACCTCGGGCTGCGAGTGGGTACGGGCAGACTAGAGTGGGTAGGGGAGATTGGAATTCCTGGTGTAGCAGGTGG
AATGCGCAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACAAAAGACTGACGCTCAGGTGCGAAAGCGTG
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CGGGGACCCCGCACAANCNGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATAT
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CATAATTNTNNANCACTNANNGNCCGNCNNTNTCTTTNATNTCNTNNACTAGAATTCCCGTTAGCANCNNNTTCNN
TATTTNTNTTCTANCGCNGTTNNNTCGANNNAATTTANGANT

Endophyte: 226

GTCNNCAGGTNGCCGTAGCTTGNCNTCCCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATTGCAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAA
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GCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGG
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TCGACT

CGCTCTGTCGTGANANGTTGANTNTTATNNGGGNGNNNNGAGCTCGTAGGGCGGTTTGTGCGGTCTGCTGTGAAATCCCGA
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CCCCNNNNANTTNTCCNTTNTTNNCTNNCNACCCCNCCNNNCANNNNGCNCNNTTCCNCTNNTNCACTTNNNNTTCT
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NTNTATTTNTNT

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CNNNNNTNTANGNNNCTCNNNNCNNNCTCNNTNNNTCNNNGNANTNCCNANNNNANNNNCCNCGNNNTAATNNNTN
NCTCCNATTACNCCNTNNTNNTTATT

GGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGTAGCTCGTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCCGAGGC
TCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC GTTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAA
TGCGCAGATATCAGGAGGAAACCCGATGGCGAAGGCCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTG
GGGAGCAAAACAGGCTTAGATACCTTGGTAGTCCACCCGATAAACGTCGTGGGAAC TAGTTGTGGGGTCCATTCCACGGATT
CCGTGACGCAGCTAACCGCATTAAGTTCCTCCGCTGGGGAGTAGCGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGCGGAGCATGCGGATTAATTTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATAGAGGAA
ACGGCTGGAAACAGTGCCTCCCGCAAGGTCTCTATACAGGTGGTGCATGGTTGTCTGCTCAGCTCGTGTCTGTGAGATGTTGG
GTAAAGTCCCGCAACGAGCGCAACCTCTGTTCTATGTTGCCAGCACGTAATGGTGGGAAC TCATGGGATACTGCCGGGG
TCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCG
GTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGTTCG GATTGAGGTCTGCAACTCG
ACCTCATGA

GTGCGATACGGTAGCCGTAAGCTTGGCTNCCGGTAAAGCGTGCCTAGGTGGTTGTTTAAAGTCTGTTGTGAAAGCCCTGGG
CTCAACCTGGGAATTGCAAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGAATCCCGGTGTAGCAGTGAA
ATGCGTAGAGATCGGGAGGAACATCCATGGCGGAAGGCAGCTACCTGGACCAACACTGACACTGAGGCCACGAAAGCGT
GGGGAGCAAAACAGGATTAGATACCTTGGTAGTCCACGCCCTAAACGATGCGAACTGGATTGGGTGCAATTTGGCAC
GAGATTCGAAGCTAACCGCTTAAGTTCCGCCCTGGGAGTAGCGGTGCGAAGACTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCCTTACCTGGTCTTGACATGTGAGAA
CTTCCAGAGATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTAGCTCGTGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTGTCTTATGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCC
GGTGACAAACCGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAAT
GTGAGGACAGAGGGTGCAAAACCCGCGAGGGCAAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCA
ACTCGACTCCATGAAGTCG

Appendix 3. The 16S rDNA contig sequence length for 220 culturable endophytic bacteria isolated from surface sterilized crown tissue from Midlawn and Tifgreen cultivars of bermudagrass. Bold type indicates the high quality sequences, > 1.0 % N content and < 500 bases long. Recal=bacterium with recalcitrant cell walls. Control=bacterial colonies that grew on sterility control agar plates.

Endophyte Identification Number	Primary	Number of Ns	Percent Ns	Secondary	Number Ns	Percent Ns
	Contig Sequence Length			Contig Sequence Length		
1	549	7	1.3	538	4	0.7
2	554	6	1.1	550	4	0.7
3	881	240	27			
4	781	133	17			
5	790	4	0.5			
6	811	27	3.3	614	4	0.7
7	543	2	0.4			
8	551	9	1.6			
9	557	6	1.1	543	2	0.4
10	800	7	0.9			
11	506	24	4.7			
12	552	1	0.2			
13	551	2	0.4			
14	780	11	1.4	742	1	0.1
15	525	20	3.8			
16	564	17	3			
17	554	6	1.1	531	5	0.9
18	551	2	0.4			
19	553	3	0.5			
20	549	4	0.7			
21	541	37	6.8			
22	550	6	1.1	538	4	0.7
23	551	2	0.4			
24	549	6	1.1	538	3	0.6
25	553	7	1.3	527	3	0.6
26	553	9	1.6	521	3	0.6
27	963	334	35			
28	554	11	2	521	3	0.6
29	560	19	3.4			
30	556	1	0.2			
31	552	18	3.3	474	1	0.2
32	785	3	0.4			
33	784	7	0.9			
34	553	2	0.4			
35	787	1	0.1			
36	531	1	0.2			

Endophyte Identification Number	Primary Contig Sequence Length	Number of Ns	Percent Ns	Secondary Contig Sequence Length	Number Ns	Percent Ns
37	550	7	1.3	522	4	0.8
38	551	6	1.1	539	4	0.7
39	554	8	1.4	533	3	0.6
40	551	2	0.4			
41	549	2	0.4			
42	551	4	0.7			
43	559	13	2.3	521	4	0.8
44	553	11	2	539	5	0.9
45	551	6	1.1	538	2	0.4
46	551	5	0.9			
47	522	25	4.8			
48	720	1	0.1			
49	522	0	0			
50	542	4	0.7			
51	551	1	0.2			
52	801	1	0.1			
53	545	0	0			
54	558	65	12			
55	536	0	0			
56	885	240	27			
57	550	2	0.4			
58	804	5	0.6			
59	550	6	1.1	534	0	0
60	555	6	1.1	548	3	0.5
61	560	16	2.9	494	3	0.6
62	554	14	2.5			
63	791	5	0.6			
64	736	18	2.4	642	5	0.9
65	555	3	0.5			
66	559	8	1.4	528	4	0.8
67	551	7	1.3	536	0	0
68	784	6	0.8			
69	793	8	1	773	0	0
70	554	5	0.9			
71	852	149	17			
72	823	143	17			
73	542	9	1.7	520	2	0.4
74	795	113	14			
75	483	9	2	429	4	0.9
76	790	2	0.2			
77	770	0	0			
78	551	4	0.7			

Endophyte Identification Number	Primary Contig Sequence Length	Number of Ns	Percent Ns	Secondary Contig Sequence Length	Number Ns	Percent Ns
79	555	7	1.3	546	5	0.9
80	554	7	1.3	532	4	0.8
81	552	3	0.5			
82	died					
83	died					
84	547	4	0.7			
85	782	0	0			
86	771	5	0.6			
87	551	3	0.5			
88	551	3	0.5			
89	553	9	1.6	542	5	0.9
90	549	9	1.6	543	5	0.9
91	549	2	0.4			
92	547	7	1.3	524	1	0.2
93	550	9	1.6	516	3	0.6
94	548	10	1.8	505	3	0.6
95	853	289	34			
96	546	3	0.5			
97	558	12	2.2			
98	890	178	2			
99	642	53	8.3			
100	783	2	0.3			
101	550	3	0.5			
102	553	3	0.5			
103	549	3	0.5			
104	791	35	4.4			
105	793	0	0			
106	549	10	1.8	536	5	0.9
107	802	113	1.4			
108	552	11	2	522	5	0.9
109	785	7	0.9			
110	790	1	0.1			
111	554	5	0.9			
112	558	6	1.1	551	3	0.5
113	521	11	2.1			
114	833	77	9.2			
115	554	5	0.9			
116	552	4	0.7			
117	865	163	18			
118	796	0	0			
119	830	171	21			
120	544	5	0.9			

Endophyte Identification Number	Primary Contig Sequence Length	Number of Ns	Percent Ns	Secondary Contig Sequence Length	Number Ns	Percent Ns
121	553	4	0.7			
122	835	133	16			
123	544	5	0.9			
124	554	3	0.5			
125	646	134	21			
126	553	3	0.5			
127	551	4	0.7			
128	552	3	0.5			
129	553	4	0.7			
130	553	5	0.9			
131	548	6	1.1	538	4	0.7
132	880	222	25			
133	804	6	0.7			
134	549	7	1.3	539	3	0.6
135	recal					
136	550	5	0.9			
137	863	123	14			
138	781	5	0.6			
139	551	5	0.9			
140	552	5	0.9			
141	552	4	0.7			
142	543	4	0.7			
143	547	1	0.2			
144	551	4	0.7			
145	549	10	1.8	494	2	0.4
146	925	176	19			
147	876	245	27			
148	555	4	0.7			
149	796	14	1.8	757	3	0.4
150	832	214	26			
151	554	5	0.9			
152	807	34	4.2	600	4	0.7
153	552	4	0.7			
154	551	8	1.5	537	4	0.7
155	542	5	0.9			
156	died					
157	538	8	1.5	506	4	0.8
158	555	6	1	540	3	0.6
159	551	2	0.4			
160	546	5	0.9			
161	552	9	1.6	515	1	0.2
162	548	8	1.5	512	1	0.2

Endophyte Identification Number	Primary Contig Sequence Length	Number of Ns	Percent Ns	Secondary Contig Sequence Length	Number Ns	Percent Ns
163	553	3	0.5			
164	543	8	1.5	508	4	0.8
165	540	1	0.2			
166	844	253	30			
167	793	9	1.1	768	5	0.7
168	790	1	0.1			
169	553	3	0.5			
170	555	4	0.7			
171	544	10	1.8	507	1	0.2
172	535	2	0.4			
173	550	9	1.6	531	4	0.8
174	549	6	1.1	535	1	0.2
175	536	3	0.6			
176	536	5	0.9			
177	550	10	1.8	527	5	0.9
178	540	1	0.2			
179	629	148	24			
180	783	1	0.1			
181	540	1	0.2			
182	546	17	3.1			
183	901	192	21			
184	540	5	0.9			
185	560	10	1.8	532	3	0.6
186	544	4	0.7			
187	540	0	0			
188	553	33	6			
189	551	3	0.5			
190	798	1	0.1			
191	491	6	1.2	477	3	0.6
192	557	5	0.9			
193	554	3	0.5			
194	Control					
195	Control					
196	542	11	2	505	3	0.6
197	560	29	5.2			
198	543	11	2	505	1	0.2
199	556	10	1.8	506	4	0.8
200	552	6	1.1	543	4	0.7
201	557	19	3.4			
202	554	8	1.4	532	5	0.9
203	788	2	0.3			
204	544	3	0.5			

Endophyte Identification Number	Primary Contig Sequence Length	Number of Ns	Percent Ns	Secondary Contig Sequence Length	Number Ns	Percent Ns
205	553	2	0.4			
206	792	6	0.8			
207	528	12	2.3	468	4	0.9
208	550	11	2	536	4	0.7
209	772	1	0.1			
210	542	9	1.7	525	4	0.8
211	773	1	0.1			
212	537	2	0.4			
213	785	10	1.3	755	2	0.3
214	563	11	2	545	4	0.7
215	539	1	0.2			
216	808	7	0.9			
217	535	3	0.7			
218	552	26	4.7			
219	794	1	0.1			
220	844	140	17			
225	496	7	1.4			
226	787	7	0.9			
227	553	11	2	514	1	0.2
228	550	42	7.6			
399	752	124	17	475	25	5
477	801	1	0.1			

Appendix 4. The putative identification and BLAST information for the 169 culturable bacterial endophytes isolated from surface sterilized crown tissue from Midlawn and Tifgreen cultivars of bermudagrass. See Appendix 5 for NCBI Accession Number references.

Endophyte Identification Number	Putative Identification	BLAST E-Value	BLAST bits	BLAST Percent Identities	NCBI Accession Numbers
1	<i>Microbacterium</i>	0.0	989	510/515 (99 %)	AF474327
2	<i>Microbacterium</i>	0.0	1025	519/520 (99 %)	AF474327
5	<i>Acidovorax</i>	0.0	1483	755/758 (99 %)	AF137506
6	<i>Afipia</i>	0.0	1209	678/695 (97 %)	U87773
7	<i>Chryseobacterium</i>	0.0	900	510/525 (97 %)	AJ457206
9	<i>Acidovorax</i>	0.0	1015	533/536 (99 %)	AF137506
10	<i>Pantoea</i>	0.0	1530	775/776 (99 %)	AF364846
12	<i>Microbacterium</i>	0.0	1029	519/519 (100 %)	AF474327
13	<i>Acidovorax</i>	0.0	1031	536/539 (99 %)	AF137506
14	<i>Acidovorax</i>	0.0	1409	726/730 (99 %)	AF137506
17	<i>Microbacterium</i>	0.0	993	517/522 (99 %)	AF474327
18	<i>Acidovorax</i>	0.0	1041	539/541 (99 %)	AF137506
19	<i>Acidovorax</i>	0.0	1037	539/542 (99 %)	AF137506
20	<i>Enterobacter/Pantoea</i>	0.0	na	na	
22	<i>Enterobacter/Pantoea</i>	0.0	na	na	
23	<i>Microbacterium</i>	0.0	1027	518/518 (100 %)	AF474327
24	<i>Enterobacter/Pantoea</i>	0.0	na	na	
25	<i>Microbacterium</i>	0.0	999	513/517 (99 %)	AF474327
26	<i>Curtobacterium</i>	0.0	977	510/515 (99 %)	AB042096
28	<i>Curtobacterium</i>	0.0	985	512/518 (98 %)	AB042096
30	<i>Pseudomonas</i>	0.0	1055	538/540 (99 %)	AJ417070
32	<i>Pseudomonas</i>	0.0	1421	746/755 (98 %)	AF388027
33	<i>Pseudomonas</i>	0.0	1465	752/757 (99 %)	AF388027
34	<i>Pseudomonas</i>	0.0	1047	538/540 (99 %)	AJ417070
35	<i>Pseudomonas</i>	0.0	1528	779/782 (99 %)	AF388027
36	<i>Chryseobacterium</i>	0.0	916	511/525 (97 %)	AJ457206
37	<i>Enterobacter/Pantoea</i>	0.0	na	na	
38	<i>Stenotrophomonas</i>	0.0	1033	521/521 (100 %)	AY040357
39	<i>Stenotrophomonas</i>	0.0	1027	518/518 (100 %)	AY040357
40	<i>Stenotrophomonas</i>	0.0	1035	536/538 (99 %)	AY040357
41	<i>Stenotrophomonas</i>	0.0	1039	534/536 (99 %)	AY040357
42	<i>Stenotrophomonas</i>	0.0	1029	535/538 (99 %)	AY040357
43	<i>Microbacterium</i>	0.0	967	496/499 (99 %)	AF474327
44	<i>Enterobacter/Pantoea</i>	0.0	na	na	
45	<i>Microbacterium</i>	0.0	1025	517/517 (100 %)	AF474327
46	<i>Stenotrophomonas</i>	0.0	674	375/380 (98 %)	AY040357
48	<i>Pseudomonas</i>	0.0	1495	763/766 (99 %)	AF388027
49	<i>Stenotrophomonas</i>	0.0	1035	536/538 (99 %)	AY040357

Endophyte Identification Number	Putative Identification	BLAST E-Value	BLAST bits	BLAST Percent Identities	NCBI Accession Numbers
50	<i>Xanthomonadaceae</i>	e-170	603	361/375 (96 %)	AY040357
51	<i>Stenotrophomonas</i>	0.0	1068	539/539 (100 %)	AY040357
52	<i>Pseudomonas</i>	0.0	1556	793/796 (99 %)	AF388027
53	<i>Pseudomonas</i>	0.0	1033	538/541 (99 %)	AJ417070
55	<i>Xanthomonas</i>	0.0	989	527/535 (98 %)	AY135649
57	<i>Stenotrophomonas</i>	0.0	1035	536/538 (99 %)	AY040357
58	<i>Rhizobium</i>	0.0	1489	765/767 (99 %)	AF531767
59	<i>Stenotrophomonas</i>	0.0	1029	533/535 (99 %)	AY040357
60	<i>Pantoea</i>	0.0	1031	534/536 (99 %)	AF364846
63	<i>Enterobacter/Pantoea</i>	0.0	na	na	
64	<i>Afipia</i>	0.0	1160	666/685 (97 %)	U87773
65	<i>Curtobacterium</i>	0.0	987	517/521 (99 %)	AB042096
66	<i>Curtobacterium</i>	0.0	965	520/528 (98 %)	AB042096
67	<i>Xanthomonadaceae</i>	e-140	505	273/279 (97 %)	AY040357
68	<i>Enterobacter/Pantoea</i>	0.0	na	na	
69	<i>Brevundimonas</i>	0.0	1532	773/773 (100 %)	AJ227780
70	<i>Microbacterium</i>	0.0	1009	518/520 (99 %)	AF474327
73	<i>Microbacterium</i>	0.0	979	515/520 (99 %)	AF474327
76	<i>Microbacterium</i>	0.0	1528	787/790 (99 %)	AF474327
77	<i>Microbacterium</i>	0.0	1491	752/752 (100 %)	AF474327
78	<i>Microbacterium</i>	0.0	1001	514/516 (99 %)	AF474327
79	<i>Microbacterium</i>	0.0	959	510/520 (98 %)	AB042073
80	<i>Curtobacterium</i>	0.0	1007	519/524 (99 %)	AB042096
81	<i>Microbacterium</i>	0.0	1013	520/522 (99 %)	AF474327
84	<i>Afipia</i>	0.0	1019	521/522 (99 %)	AJ300771
85	" <i>Betaproteobacteria</i> "	0.0	1515	718/784 (99 %)	AF423075
86	" <i>Betaproteobacteria</i> "	0.0	1461	764/772 (98 %)	AF423075
87	<i>Staphylococcus</i>	0.0	1055	532/532 (100 %)	AF540985
88	<i>Staphylococcus</i>	0.0	1029	519/519 (100 %)	AF540985
89	<i>Microbacterium</i>	0.0	997	517/521 (99 %)	AY082800
90	<i>Brevundimonas</i>	0.0	1013	513/514 (99 %)	AJ227781
91	<i>Brevundimonas</i>	0.0	1029	526/527 (99 %)	AJ227781
92	<i>Rhizobium</i>	0.0	795	489/517 (94 %)	Z79620
93	<i>Microbacterium</i>	0.0	973	503/506 (99 %)	AF474327
94	<i>Microbacterium</i>	0.0	975	498/501 (99 %)	AF474327
96	" <i>Alphaproteobacteria</i> "	e-165	587	367/379 (96 %)	AF445712
100	<i>Microbacterium</i>	0.0	1398	745/752 (99 %)	AF474327
101	" <i>Alphaproteobacteria</i> "	0.0	922	523/537 (97 %)	AF445712
102	<i>Microbacterium</i>	0.0	1025	517/517 (100 %)	AY082800
103	<i>Brevundimonas</i>	0.0	1037	530/531 (99 %)	AJ227781
105	" <i>Betaproteobacteria</i> "	0.0	1540	790/793 (99 %)	AF423075
106	<i>Xanthomonas</i>	0.0	955	510/518 (98 %)	AY135649
108	<i>Microbacterium</i>	0.0	979	515/522 (98 %)	AY082800

Endophyte Identification Number	Putative Identification	BLAST E-Value	BLAST bits	BLAST Percent Identities	NCBI Accession Numbers
109	<i>"Betaproteobacteria"</i>	0.0	1467	752/755 (99 %)	AF423075
110	<i>Microbacterium</i>	0.0	1507	762/763 (99 %)	AF474327
111	<i>Brevundimonas</i>	0.0	1015	514/515 (99 %)	AJ227781
112	<i>"Alphaproteobacteria"</i>	0.0	902	527/545 (96 %)	AF445712
115	<i>Brevundimonas</i>	0.0	1021	515/515 (100 %)	AJ227781
116	<i>Curtobacterium</i>	0.0	1013	522/525 (99 %)	AB042096
118	<i>Acidovorax</i>	0.0	1552	790/791 (99 %)	AF137506
120	<i>Microbacterium</i>	0.0	983	511/516 (99 %)	AF474327
121	<i>Sphingomonas</i>	0.0	1001	513/516 (99 %)	AF131295
123	<i>"Alphaproteobacteria"</i>	0.0	1005	509/510 (99 %)	AF288308
124	<i>"Alphaproteobacteria"</i>	0.0	1015	512/512 (100 %)	AF288308
126	<i>Acidovorax</i>	0.0	1039	524/524 (100 %)	AF137506
127	<i>Acidovorax</i>	0.0	1025	517/517 (100 %)	AF137506
128	<i>Acidovorax</i>	0.0	1033	535/537 (99 %)	AF137506
129	<i>"Betaproteobacteria"</i>	0.0	1017	516/517 (99 %)	AF423075
130	<i>Acidovorax</i>	0.0	1033	523/524 (99 %)	AF137506
131	<i>Microbacterium</i>	0.0	1021	515/515 (100 %)	AF474327
133	<i>Klebsiella</i>	0.0	1517	768/770 (99 %)	AF511429
134	<i>Curtobacterium</i>	0.0	1027	518/518 (100 %)	AY273208
136	<i>Microbacterium</i>	0.0	991	513/516 (99 %)	Y17238
138	<i>Microbacterium</i>	0.0	1495	754/745 (100 %)	AF474327
139	<i>Microbacterium</i>	0.0	1029	519/519 (100 %)	AF474327
140	<i>Microbacterium</i>	0.0	1033	521/521 (100 %)	AF474327
141	<i>Microbacterium</i>	0.0	1023	518/519 (99 %)	AY082800
142	<i>Microbacterium</i>	0.0	981	513/519 (98 %)	AF474327
143	<i>Geodermatophilus</i>	0.0	912	523/540 (96 %)	L40620
144	<i>Microbacterium</i>	0.0	1033	521/521 (100 %)	AY082800
148	<i>Microbacterium</i>	4E-22	113	125/151 (82 %)	AB004725
149	<i>Microbacterium</i>	0.0	1471	746/748 (99 %)	AF474327
151	<i>Acitnobacteria</i>	0.0	924	507/521 (97 %)	AY048891
152	<i>Microbacterium</i>	0.0	1235	702/729 (96 %)	AF474327
153	<i>Amycolatopsis</i>	0.0	997	519/522 (99 %)	AY129777
154	<i>Microbacterium</i>	0.0	1019	516/517 (99 %)	AF474327
155	<i>Amycolatopsis</i>	0.0	999	504/504 (100 %)	AY129777
157	<i>Microbacterium</i>	0.0	967	511/519 (98 %)	AY082800
158	<i>Microbacterium</i>	0.0	1003	527/532 (99 %)	AF474327
159	<i>Microbacterium</i>	0.0	1029	519/519 (100 %)	AY082800
160	<i>Microbacterium</i>	0.0	1015	519/520 (99 %)	AF474327
161	<i>Microbacterium</i>	0.0	997	505/507 (99 %)	AY082800
162	<i>Microbacterium</i>	0.0	999	511/512 (99 %)	AF474327
163	<i>Microbacteriaceae</i>	0.0	971	515/522 (98 %)	AB028941
164	<i>Microbacterium</i>	0.0	928	488/494 (98 %)	AY082800
165	<i>Microbacterium</i>	0.0	1029	529/531 (99 %)	AY082800

Endophyte Identification Number	Putative Identification	BLAST E-Value	BLAST bits	BLAST Percent Identities	NCBI Accession Numbers
167	<i>Microbacterium</i>	0.0	1404	747/757 (98 %)	AF474327
168	<i>Chryseobacterium</i>	0.0	1443	774/788 (98 %)	AJ457206
169	<i>Microbacterium</i>	0.0	1021	522/532 (99 %)	AF474327
170	<i>Curtobacterium</i>	0.0	1017	516/517 (99 %)	AB042096
171	<i>Microbacterium</i>	0.0	959	501/507 (98 %)	AJ244679
172	<i>Xanthomonas</i>	0.0	967	509/516 (98 %)	AY135649
173	<i>Curtobacterium</i>	0.0	1001	514/518 (99 %)	AF474329
174	<i>Microbacterium</i>	0.0	1029	519/519 (100 %)	AF474327
175	<i>Mycobacterium</i>	0.0	995	527/532 (99 %)	AF480582
176	<i>Microbacterium</i>	0.0	1011	514/516 (99 %)	AY082800
177	<i>Microbacterium</i>	0.0	991	513/519 (98 %)	AF474327
178	<i>Mycobacterium</i>	0.0	1015	515/516 (99 %)	AF480593
180	<i>Microbacterium</i>	0.0	1546	782/783 (99 %)	AF474327
181	<i>Microbacterium</i>	0.0	1025	517/517 (100 %)	AY082800
184	<i>Microbacterium</i>	0.0	1003	512/515 (99 %)	AF474327
185	<i>Acidovorax</i>	0.0	963	503/508 (99 %)	AF137506
186	<i>Microbacterium</i>	0.0	1011	517/518 (99 %)	AY082800
187	<i>Microbacterium</i>	0.0	1031	520/520 (100 %)	AF474327
189	<i>Microbacterium</i>	0.0	1017	517/519 (99 %)	AY082800
190	<i>Microbacterium</i>	0.0	1499	762/746 (99 %)	Y17238
192	<i>"Alphaproteobacteria"</i>	0.0	1027	518/518 (100 %)	AF288308
193	<i>"Alphaproteobacteria"</i>	0.0	1029	519/519 (100 %)	AF288308
196	<i>Microbacterium</i>	0.0	948	498/503 (99 %)	AF474327
198	<i>Microbacterium</i>	0.0	995	504/505 (99 %)	AF474327
199	<i>Microbacterium</i>	0.0	872	425/428 (99 %)	AF474327
200	<i>Microbacterium</i>	0.0	1009	518/520 (99 %)	AF474327
202	<i>Microbacterium</i>	0.0	989	504/506 (99 %)	AF474327
203	<i>Microbacterium</i>	0.0	1469	748/749 (99 %)	AF474327
204	<i>Microbacterium</i>	0.0	1009	520/523 (99 %)	AF474327
205	<i>Microbacterium</i>	0.0	1023	518/519 (99 %)	AF474327
206	<i>Microbacterium</i>	0.0	1429	737/744 (99 %)	AF474327
208	<i>Bacillus</i>	0.0	1023	516/516 (100 %)	AY112667
209	<i>Microbacterium</i>	0.0	1503	770/773 (99 %)	AF474327
210	<i>Microbacterium</i>	0.0	955	491/493 (99 %)	AF474327
211	<i>Microbacterium</i>	0.0	1517	767/768 (99 %)	AF474327
212	<i>Bacillus</i>	0.0	1039	533/535 (99 %)	AY112667
213	<i>Klebsiella</i>	0.0	1473	752/754 (99 %)	AB074192
214	<i>Microbacterium</i>	0.0	965	508/515 (98 %)	AF474327
215	<i>Klebsiella</i>	0.0	1053	538/539 (99 %)	AB074192
216	<i>Klebsiella</i>	0.0	1532	799/807 (99 %)	AB074192
217	<i>Microbacterium</i>	0.0	1166	681/709 (96 %)	AF474327
219	<i>Bacillus</i>	0.0	1554	791/792 (99 %)	AY144451
226	<i>Klebsiella</i>	0.0	1477	756/759 (99 %)	AB074192

Endophyte Identification Number	Putative Identification	BLAST E-Value	BLAST bits	BLAST Percent Identities	NCBI Accession Numbers
227	<i>Microbacterium</i>	0.0	1031	513/514 (99 %)	AF474327
477	<i>Stenotrophomonas</i>	0.0	1526	770/770 (100 %)	AY040357

Appendix 5. National Center for Biotechnology Information (NCBI) accession numbers used in the putative identification of culturable bacterial endophytes isolated from the crown tissue of Midlawn and Tifgreen cultivars of bermudagrass and type species references.

NCBI

Accession

Number	Name	Reference
Culturable Bacterial Endophytes		
AY513502	<i>Escherichia coli</i> 0157 : H7	Gee et al. 2004
AY048891	Uncultured bacterium	Heuer et al. 2002
AF137506	<i>Acidovorax avenae</i> subsp. <i>citrulli</i>	Hu et al. 2001
U87773	<i>Afipia</i> genosp. 7	Whitney 1997
AJ300771	<i>Afipia</i> sp.	Mergaert et al. 2001
AF445712	Uncultured alpha proteobacterium	Bonheyo et al. 2001
AF288308	Uncultured alpha proteobacterium	La Scola et al. 2000
AY129777	<i>Amycolatopsis</i> sp.	Tan et al. 2002
AY144451	<i>Bacillus megaterium</i>	Xu et al. 2002
AY112667	<i>Bacillus pumilus</i>	Isenegger et al. 2003
AF423075	Beta proteobacterium	Pitulle et al. 2001
AJ227780	<i>Brevundimonas vesicularis</i>	Abraham et al. 1999
AJ227781	<i>Brevundimonas vesicularis</i>	Abraham et al. 1999
AJ457206	<i>Chryseobacterium</i> sp.	Kim and Lee 2002
AB042096	<i>Curtobacterium</i> sp.	Evtushenko et al. 2000
AY273208	<i>Curtobacterium flaccumfaciens</i> pv. <i>beticola</i>	Chen et al. 2003
AF474329	<i>Curtobacterium</i> sp.	Zinniel et al. 2002
AF395913	<i>Enterobacter aerogenes</i>	Yu et al. 2001
AJ002811	<i>Pantoea</i> sp.	Hoffmann et al. 1998
L40620	<i>Geodermatophilus obscurus</i>	Normand et al. 1996
AB074192	<i>Klebsiella</i> sp.	Fukuda et al. 2001
AF511429	<i>Klebsiella pneumoniae</i>	Ovesen et al. 2002
AB028941	<i>Microbacteriaceae</i>	Suzuki et al. 1999
AF474327	<i>Microbacterium testaceum</i>	Zinniel et al. 2002
Y17238	<i>Microbacterium terrae</i>	Schumann et al. 1999
AB004725	<i>Microbacterium chocolatum</i>	Takeuchi and Hatano 1998
AB042073	<i>Microbacterium</i> sp.	Evtushenko et al. 2000
AY082800	<i>Microbacterium</i> sp.	Lau et al. 2002

NCBI Accession Number	Name	Reference
AJ244679	<i>Microbacterium</i> sp.	Fritz 1999
AF480582	<i>Mycobacterium lacticola</i>	Turenne et al. 2001
NCBI		
AF480593	<i>Mycobacterium neoaurum</i>	Turenne et al. 2001
AF364846	<i>Pantoea ananatis</i>	Coutinho et al. 2001
AJ417070	<i>Pseudomonas</i> sp.	Ramette et al. 2001
AF388027	<i>Pseudomonas</i> sp.	Macur et al. 2004
Z79620	<i>Rhizobium galegae</i>	Huber and Selenska-Pobell 1994
AY174112	<i>Agrobacterium</i> sp. (=Rhizobium)	Trott et al. 2003
AF131295	<i>Sphingomonas</i> sp.	Lee et al. 2001
AF540985	<i>Staphylococcus epidermidis</i>	Xu et al. 2002
AY040357	<i>Stenotrophomonas maltophilia</i>	Goris et al. 2001
AY135649	<i>Xanthomonas axonopodis</i> pv. <i>allii</i>	Roumagnac et al. 2004
Type Species		
AF420324	<i>Acidovorax facilis</i>	Swiderski 2001
AF338177	<i>Afipia felis</i>	van Berkum and Eardly 2002
X76958	<i>Amycolatopsis orientalis</i>	Warwick et al. 1993
X60646	<i>Bacillus subtilis</i>	Ash et al. 1991
M59064	<i>Pseudomonas diminuta</i>	Woese 1991
AY468449	<i>Chryseobacterium glem</i>	Matte-Tailliez et al. 2003
X77436	<i>Curtobacterium citreum</i>	Rainey et al. 1994
AJ251469	<i>Enterobacter cloacae</i>	Boye and Hansen 1999
X92356	<i>Geodermatophilus obscurus</i>	Eppard et al. 1996
AF130981	<i>Kelbsiella pneumoniae</i>	Drancourt et al. 2001
D21343	<i>Microbacterium lacticum</i>	Takeuchi and Yokota 1994
X55588	<i>Mycobacterium tuberculosis</i>	Wolters 1990
AF130953	<i>Enterobacter agglomerans</i>	Rojas et al. 1999
Z76672	<i>Pseudomonas aeruginosa</i>	Moore et al. 1996
AY509899	<i>Rhizobium leguminosarum</i>	Valverde et al. 2003
U37337	<i>Sphingomonas pauchimobilis</i>	Mueller et al. 1997
D83357	<i>Staphylococcus aureus</i>	Takahashi et al. 1996
M59158	<i>Xanthomonas</i>	Woese 1990
	(=Stenotrophomonas) maltophilia	
X95917	<i>Xanthomonas campestris</i>	Moore et al. 1997

Appendix 6. Full taxonomic genera names of putatively identified culturable bacterial endophytes. Synonyms published prior to 1970 were not included. (NCBI Taxonomic List, 2004, J. P. Euzéby 2005 (www.bacterio.cict.fr)).

Acidovorax Willems et al. 1990 emend. Willems et al. 1992

Equivalent name: *Acidivorax*

Afipia Brenner et al. 1992 emend. La Scola et al. 2002

Amycolatopsis Lechevalier et al. 1986

Bacillus Cohn 1872

Brevundimonas Segers et al. 1994 emend. Abraham et al. 1999

Chryseobacterium Vandamme et al. 1994

Curtobacterium Yamada and Komagata 1972

Equivalent name: *Curtibacterium*

Enterobacter Hormaeche and Edwards 1960

Geodermatophilus Luedemann 1968

Klebsiella Trevisan 1885 emend. Drancourt et al. 2001

Microbacterium Orla-Jensen 1991 emend. Takeuchi and Hatano 1998

Synonym: *Aureobacterium*

Equivalent name: *Aureibacterium*

Mycobacterium Lehmann and Neumann 1896

Pantoea Gavini et al. 1989 emend. Mergaert et al. 1993

Pseudomonas Migula 1894

Rhizobium Frank 1889 emend. Young et al. 2001

Synonym: *Rhizobacterium*, *Phytomyxa*

Sphingomonas Yabuuchi et al. 1990 emend. Busse et al. 2003

Staphylococcus Rosenbach 1884

Synonym: *Aurococcus*

Stenotrophomonas Palleroni and Bradbury 1993

Xanthomonas Dowson 1939 emend. Vauterin et al. 1995

Synonym: “*Phytomonas*”

Appendix 7. Indistinguishable and distinguishable 16S rDNA contig sequences of putatively identified *Microbacterium*. The bold numbers designate the randomly chosen set representative.

Indistinguishable

Set 1	17 , 177
Set 2	202, 214
Set 3	100, 160 , 162
Set 4	102, 141, 144 , 159, 161, 176, 181, 189
Set 5	2, 12, 23, 45, 70, 76, 77, 78, 81, 94, 110, 120, 131, 138, 139, 140, 149, 154, 167, 169, 174, 180, 184, 187, 198, 200 , 204, 205, 209, 211, 277

Distinguishable

Set A	1, 25, 43, 73, 79, 89, 93, 108, 136, 142, 148, 152, 157, 158, 164, 165, 171, 186, 190, 196, 199, 203, 206, 210, 217
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Appendix 8. Bacterial endophytes documented in other studies and also documented in this study of the crown tissue of Midlawn and Tifgreen cultivars of bermudagrass.

Bacterial Endophyte	Plant	Organ	Reference
<i>Acidovorax</i>	Potato	Tuber	Sturz et al. 1999
	Clover	Roots	Sturz et al. 1998
	Potato	Tuber	Sturz et al. 1998
<i>Afipia</i>	Potato	Seedlings	Garbeva et al. 2001
<i>Bacillus</i>	Aspen	Wood	Knutson 1973
	Canola	Root	Germida et al. 1998
	Citrus	Branch	Araujo et al. 2002
	Citrus	Leaf	Araujo et al. 2001
	Clover	Root	Sturz et al. 1998
	Corn	Root	McInroy and Kloepper 1995a
	Corn	Kernel	Bacon and Hinton 2002
	Cotton	Plant tissues	Chen et al. 1995
	Live oak	Plant tissues	Brooks et al. 1994
	Sorghum	Stem	Zinniel et al. 2002
	Cotton	Seedlings	Zhao and Ma 1999
	Cotton	Root	Misaghi and Donndelinger 1990
	Elm	Stem, root	Mocali et al. 2003
	Grapevine	Xylem	Bell et al. 1995
	Maple	Stem	Hall et al. 1986
	Lemon	Root	Gardner et al. 1982
	Oilseed rape	Seedling	Graner et al. 2003
	Pea	Stem	Elvira-Recuenco & van Vuurde 2000
	Potato	Tuber	Lutman and Wheeler 1948
	Potato	Plant tissues	De Boer and Copeman 1974
	Potato	Tuber	Sturz et al. 1998
	Red Clover	Root, nodule	Sturz et al. 1997
	Rice	Plant tissues	Liu et al. 1999
	<i>Sinapis</i>		Pleban et al. 1997
	Sugar beet	Root	Jacobs et al. 1985
	Wheat	Leaf	Larran et al. 2002
	Wheat	Root	Germida and Siciliano 2001
	Vegetables	Plant tissues	Meneley and Stanghellini 1972
	27 plants	Ovule, seed	Mundt and Hinkle 1976
<i>Brevundimonas</i>	Potato	Seedlings	Garbeva et al. 2001
<i>Burkholderia cepacia</i>	Banana	Root	Pan et al. 1997
<i>(Pseudomonas cepacia)</i>	Cotton	Plant tissues	Chen et al. 1995
<i>Curtobacterium</i>	Canola	Root	Germida et al. 1998
	Citrus	Branch	Araujo et al. 2002
	Citrus	Leaf	Araujo et al. 2001
	Clover	Root	Sturz et al. 1998
	Corn	Root	McInroy and Kloepper 1995b
	Corn	Stem	Zinniel et al. 2002

Bacterial Endophyte	Plant	Organ	Reference
<i>Curtobacterium</i>	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Root	McInroy and Kloepper 1995
	Elm	Stem, root	Mocali et al. 2003
	Grapevine	Xylem	Bell et al. 1995
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998
	Red Clover	Root	Sturz and Christie 1998
	Red Clover	Root, nodule	Sturz et al. 1997
	Rice	Root	Germida and Siciliano 2001
	Sorghum	Stem	Zinniel et al. 2002
	Wheat	Root	Germida and Siciliano 2001
	Yam	Tuber	Mantell 1998
	200 plant species	Leaf	Dunleavy 1989
<i>Enterobacter</i>	Citrus	Leaf	Araujo et al. 2001
	Citrus	Branch	Araujo et al. 2002
	Clover	Root	Sturz et al. 1998
	Corn	Stem	Zinniel et al. 2002
	Corn	Root	Hinton and Bacon 1995
	Corn	Stem	Fisher et al. 1992
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Root	McInroy and Kloepper 1995a
	Elm	Stem, root	Mocali et al. 2003
	Grapevine	Xylem	Bell et al. 1995
	Lemon	Root	Gardner et al. 1982
	Potato	Tuber	Sturz et al. 1998
	Potato	Seedlings	Garbeva et al. 2001
	Red Clover	Plant tissues	Sturz et al. 1997
	Red Clover	Root	Sturz and Christie 1998
	Rice	Root	Yang et al. 1999
	Rice	Seedlings	Mukhopadhyay et al. 1996
	Sorghum	Stem	Zinniel et al. 2002
	Spinach	Root	Tsuda et al. 2001
	Wheat	Root	Germida and Siciliano 2001
	Yam	Tuber	Omoregie et al. 1999
	Yam	Tuber	Mantell 1998
	27 plants	Ovule, seed	Mundt and Hinkle 1976
<i>Klebsiella</i>	Clover	Root	Sturz et al. 1998
	Corn	Root	Palus et al. 1996
	Corn	Root	Riggs et al. 2001
	Corn	Stem	Zinniel et al. 2002
	Corn	Root	Chelis and Triplett 2000
	Corn	Stem	Fisher et al. 1992
	Cotton	Root	McInroy and Kloepper 1995
	Grapevine	Xylem	Bell et al. 1995

Bacterial Endophyte	Plant	Organ	Reference
<i>Klebsiella</i>	Lemon	Root	Gardner et al. 1982
	Potato	Tuber	Sturz et al. 1998
	Rice	Stem, seed	Elbeltagy et al. 2000
	Rice	Root	Englehard et al. 2000
	Sorghum	Stem	Zinniel et al. 2002
<i>Microbacterium</i>	Corn	Root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Potato	Seedlings	Garbeva et al. 2001
	Sorghum	Stem	Zinniel et al. 2002
<i>Aureobacterium</i> <i>synonym Microbacterium</i>	Cotton	Plant tissues	Chen et al. 1995
<i>Mycobacterium</i>	Truifgrass	Seed, root	Sundaram et al. 1988
<i>Pantoea</i>	Scots pine	Bud	Mattila 2001
	Citrus	Leaf	Araujo et al. 2001
	Citrus	Branch	Araujo et al. 2002
	Clover	Root	Sturz et al. 1998
	Corn	Root	Riggs et al. 2001
	Cotton	Root	McInroy and Kloepper 1995
	Grapevine	Xylem	Bell et al. 1995
	Oilseed rape	Seedlings	Graner et al. 2003
	Pea	Stem	Elvira-Recuenco & van Vuurde 2000
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998
	Potato	Tuber	Sturz and Matheson 1996
	Red Clover	Root, nodule	Sturz et al. 1997
	Rice	Root	Verma et al. 2001
	Rice	Stem, seed	Elbeltagy et al. 2000
	Wheat	Root	Ruppel et al. 1992
	Yam	Tuber	Omoregie et al. 1999
	Nonleguminous plants	Apoplast	Hecht-Buchholz 1998
<i>Pseudomonas</i>	Alfalfa	Root	Gagne et al. 1987
	Canola	Root	Misko and Germida 2002
	Carrots	Tuber	Surette et al. 2003
	Cherry	Plant tissues	Cameron 1970
	Clover	Root	Sturz et al. 1998
	Corn	Root	McInroy and Kloepper 1995
	Corn	Stem	Zinniel et al. 2002
	Corn	Stem	Fisher et al. 1992
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Root	McInroy and Kloepper 1995
	Cotton	Seedlings	Zhao and Ma 1999
	Elm	Stem, root	Mocali et al. 2003
	Grapevine	Xylem	Bell et al. 1995
	Lemon	Root	Gardner et al. 1982
	Live oak	Plant tissues	Brooks et al. 1994

Bacterial Endophyte	Plant	Organ	Reference
<i>Pseudomonas</i>	Oilseed rape	Seedlings	Graner et al. 2003
	Onion	Root	Barka et al. 2002
	Pea	Stem	Elvira-Recuenco & van Vuurde 2000
	Pear	Stem,root	Whitesides and Spotts 1991
	Potato	Plant tissues	De Boer and Copeman 1974
	Potato	Seedlings	Garbeva et al. 2001
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998
	Red Clover	Root	Sturz and Christie 1998
	Red Clover	Root, nodule	Sturz et al. 1997
	Rice	Root	Yang et al. 1999
	Rice	Stem, root	Adhikari et al. 2001
	Scots pine	Buds	Mattila 2001
	Sorghum	Stem	Zinniel et al. 2002
	Sugar beet	Root	Jacobs et al. 1985
	Wheat	Root	Germida and Siciliano 2001
	Yam	Tuber	Mantell 1998
	Vegetables	Plant tissues	Meneley and Stanghellini 1972
	27 plants	Ovule, seed	Mundt and Hinkle 1976
<i>Rhizobium</i>	Carrots	Tuber	Surette et al. 2003
	Clover	Root	Philipson and Blair 1957
	Corn	Root	McInroy and Kloepper 1995
	Corn	Stem	Zinniel et al. 2002
	Cotton	Root	McInroy and Kloepper 1995
	Potato	Tuber	Sturz et al. 1999
	Potato	Seedlings	Garbeva et al. 2001
	Red Clover	Root, nodule	Sturz et al. 1997
	Red Clover	Root	Struz and Christie 1998
	Rice	Root	Yang et al. 1999
	Sorghum	Stem	Zinniel et al. 2002
	Wheat	Root	Germida and Siciliano 2001
	Nonlegumin- ous plants	Apoplast	Hecht-Buchholz 1998
<i>Sphingomonas</i>	Clover	Root	Sturz et al. 1998
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Root	McInroy and Kloepper 1995
	Elm	Stem, root	Mocali et al. 2001
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998
	Red clover	Root	Sturz and Christie 1998
	Rice	Plant tissues	Elbeltagy et al. 2000
	Rice	Root	Englehard et al. 2000
	Rice	Stem, root	Adhikari et al. 2001

Bacterial Endophyte	Plant	Organ	Reference
<i>Staphylococcus</i>	Canola	Root	Germida et al. 1998
	Carrots	Tuber	Surette et al. 2003
	Corn	Root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Root	McInroy and Kloepper 1995
	Elm	Stem, root	Mocali et al. 2003
	Grapevine	Xylem	Bell et al. 1995
	Rice	Root	Yang et al. 1999
<i>Stenotrophomonas</i>	Corn	Root	McInroy and Kloepper 1995
	Elm	Stem, root	Mocali et al. 2003
	Potato	Plant tissues	Garbeva et al. 2001
<i>Xanthomonas</i>	Citrus	Branch	Araujo et al. 2002
	Clover	Root	Sturz et al. 1998
	Corn	Root	McInroy and Kloepper 1995
	Corn	Stem	Zinniel et al. 2002
	Cotton	Seedlings	Zhao and Ma 1999
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Root	Misaghi and Donndellinger 1990
	Grapevine	Xylem	Bell et al. 1995
	Mulberry	Shoot	Sato et al. 2000
	Pinto beans	Stem	Thomas and Graham 1948
	Potato	Plant tissues	De Boer and Copeman 1974
	Potato	Tuber	Sturz et al. 1998
	Potato	Tuber	Sturz et al. 1999
	Red Clover	Plant tissues	Struz et al. 1997
	Red Clover	Root	Sturz and Christie 1998
	Rice	Root	Germida and Siciliano 2001
	Rice	Root	Yang et al. 1999
	Sorghum	Stem	Zinniel et al. 2002
	Sugar beet	Root	Jacobs et al. 1985
	Wheat	Root	Germida and Siciliano 2001
	Yam	Tuber	Mantell 1998
	Vegetables	Plant tissues	Meneley and Stanghellini 1972
	27 plants	Ovule, seed	Mundt and Hinkle 1976

Appendix 9. Bacterial endophytes documented in other studies but not documented in this study of the crown tissue from Midlawn and Tifgreen cultivars of bermudagrass.

Bacterial Endophyte	Plant	Organ	Reference
<i>Acetobacter</i>	Pineapple	Plant tissues	Tapia et al. 2000
	Sugarcane	Stem	Dong et al. 1994
<i>Acetobacterium</i>	Sea grass	Cortex	Kusel et al. 1999
<i>Achromobacter</i>	Citrus	Xylem	Gardner et al. 1982
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Acinetobacter</i>	Citrus	Xylem	Gardner et al. 1982
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem	McInroy and Kloepper 1995
	Potato	Tuber	Sturz et al. 1998
	Nonleguminous plants	Apoplast	Hecht-Buchholz 1998
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Actinomyces</i>	Citrus	Xylem	Gardner et al. 1982
<i>Aerococcus</i>	Cotton	Plant tissues	Chen et al. 1995
<i>Aeromonas</i>	Rice	Root	Yang et al. 1999
<i>Agrobacterium</i>	Carrot	Plant tissues	Surette et al. 2003
	Clover	Root	Sturz et al. 1998
	Cotton	Plant tissues	Chen et al. 1995
	Healthy rose	Plant tissues	Marti et al. 1999
	Potato	Plant tissues	De Boer and Copeman 1974
	Potato	Tuber	Sturz et al. 1998
	Red clover	Root	Struz and Christie 1998
	Rice	Root	Germida and Siciliano 2001
	Citrus	Branch	Araujo et al. 2002
	Citrus	Leaf	Araujo et al. 2001
<i>Alcaligenes</i>	Cotton	Root	McInroy and Kloepper 1995
	Oliseed rape	Seedling	Graner et al. 2003
	Potato	Tuber	Sturz et al. 1998
	Red clover	Root	Sturz and Christie 1998
	Rice	Root	Yang et al. 1999
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
	Canola	Root	Germida et al. 1998
<i>Arthrobacter</i>	Citrus	Xylem	Gardner et al. 1982
	Clover	Root	Sturz et al. 1998
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem	McInroy and Kloepper 1995
	Potato	Tuber	Sturz et al. 1998
	Potato	Tuber	Sturz et al. 1999
	Rice	Root	Germida and Siciliano 2001
	Red clover	Root	Sturz and Christie 1998

Bacterial Endophyte	Plant	Organ	Reference
<i>Azoarcus</i>	Rice	Root	Englehard et al. 2000
	Nonleguminous plants	Apoplast	Hecht-Buchholz 1998
<i>Azorhizobium</i>	Rice	Root	Englehard et al. 2000
<i>Azospirillum</i>	Rice	Plant tissues	Elbeltagy et al. 2000
	Rice	Root	Englehard et al. 2000
	Nonleguminous plants	Apoplast	Hecht-Buchholz 1998
<i>Bortedella</i>	Clover	Root	Sturz et al. 1998
	Red clover	Nodule	Sturz et al. 1997
<i>Brandyrhizobium</i>	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
<i>Brevibacterium</i>	Cotton	Plant tissues	Chen et al. 1995
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Burkholderia</i>	Banana	Plant tissues	Pan et al. 1997
	Citrus	Branch	Araujo et al. 2002
	Citrus	Leaf	Araujo et al. 2001
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Lupine	Shoot, root	Baldandrea et al. 2001
	Rice	Root	Englehard et al. 2000
	Sugarcane	Plant tissues	Boddey et al. 2003
	Wheat	Shoot, root	Baldandrea et al. 2001
	Clover	Root	Sturz et al. 1998
	Red clover	Root	Sturz and Christie 1998
<i>Cedecea</i>	Cotton	Plant tissues	Chen et al. 1995
<i>Cellulomonas</i>	Clover	Root	Sturz et al. 1998
	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
	Cotton	Stem, root	McInroy and Kloepper 1995
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998
<i>Chryseomonas</i>	Cotton	Plant tissues	Chen et al. 1995
	Rice	Root	Yang et al. 1999
<i>Citrobacter</i>	Citrus	Xylem	Gardner et al. 1982
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
<i>Clavibacter</i>	Corn	Stem, root	McInroy and Kloepper 1995
	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
	Grapevine	Xylem	Bell et al. 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Cotton	Whole plant	Misaghi and Donndelinger 1990
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998
	Rice	Root	Germida and Siciliano 2001

Bacterial Endophyte	Plant	Organ	Reference
<i>Clostridium carbonei</i>	Pinto beans	Plant tissues	Thomas and Graham 1952
<i>Comamonas</i>	Clover	Root	Sturz et al. 1998
	Cotton	Root	McInroy and Kloepper 1995
	Grapevine	Xylem	Bell et al. 1995
	Oilseed rape	Seedling	Graner et al. 2003
	Potato	Tuber	Sturz et al. 1998
	Red clover	Root	Sturz et al. 1997
<i>Corynebacterium</i>	Citrus	Xylem	Gardner et al. 1982
	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
	Cotton	Plant tissues	Chen et al. 1995
	Pinto Beans	Stem	Thomas and Graham 1952
	Rice	Plant tissues	Elbeltagy et al. 2000
	Sugar beet	Root	Jacobs et al. 1985
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Curtobacter</i>	Canola	Root	Germida et al. 1998
<i>Cytophaga</i>	Rice	Root	Germida and Siciliano 2001
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Cytophagales</i>	Rice	Plant tissues	Elbeltagy et al. 2000
<i>Deleya</i>	Clover	Root	Sturz et al. 1998
	Potato	Tuber	Sturz et al. 1998
<i>Desulfovibrio</i>	Sea grass	Cortex	Kusel et al. 1999
<i>Erwinia</i>	Aspen	Wood	Knutson 1973
	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
	Cotton	Plant tissues	Misaghi and Donndelinger 1990
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Cotton	Seedlings	Zhao and Ma 1999
	Oilseed rape	Seedlings	Graner et al. 2003
	Potato	Tuber	Sturz et al. 1998
	Red clover	Root	Sturz and Christie 1998
	Rice	Root	Germida and Siciliano 2001
	Sugar beet	Root	Jacobs et al. 1985
	Vegetables	Plant tissues	Meneley and Stanghellini 1972
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Escherichia</i>	Corn	Stem, root	McInroy and Kloepper 1995
	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
	Cotton	Stem, root	McInroy and Kloepper 1995
	Red clover	Stem	Sturz et al. 1997
<i>Flavimonas</i>	Corn	Root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
<i>Flavobacterium</i>	Canola	Root	Germida et al. 1998
	Citrus	Xylem	Gardner et al. 1982
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Root	McInroy and Kloepper 1995

Bacterial Endophyte	Plant	Organ	Reference
<i>Flavobacterium</i>	Potato	Plant tissues	De Boer and Copeman 1974
	Potato	Tuber	Sturz et al. 1998
	Potato	Stem, root	Garbeva et al. 2001
	Red clover	Root	Sturz and Christie 1998
	Rice	Plant tissues	Elbeltagy et al. 2000
	Rice	Root	Germida and Siciliano 2001
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Gallionella</i>	Rice	Root	Englehard et al. 2000
<i>Gluconacetobacter</i>	Sugarcane	Plant tissues	Boddey et al. 2003
<i>Herbaspirillum</i>	Rice	Plant tissues	Elbeltagy et al. 2000
	Rice	Root	Englehard et al. 2000
	Sugarcane	Plant tissues	Boddey et al. 2003
	Nonleguminous plants	Apoplast	Hecht-Buchholz 1998
<i>Hydrogenophaga</i>	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
<i>Kingella</i>	Potato	Tuber	Sturz et al. 1999
	Red clover	Root	Sturz and Christie 1998
<i>Kluyvera</i>	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Root	McInroy and Kloepper 1995
<i>Kurtia</i>	Oilseed rape	Seedlings	Graner et al. 2003
<i>Lactobacillus</i>	Sugar beet	Root	Jacobs et al. 1985
<i>Leuconostoc</i>	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Methylobacterium</i>	Citrus	Branch	Araujo et al. 2002
	Citrus	Leaf	Araujo et al. 2001
	Clover	Root	Sturz et al. 1998
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Red clover	Root	Sturz et al. 1997
	Scots pine	Plant tissues	Mattila 2001
	Cereal plants	Plant tissues	Coombs et al. 2003
	Corn	Leaf	de Araujo et al. 2000
	Canola	Root	Germida et al. 1998
<i>Microbispora</i>	Clover	Root	Sturz et al. 1998
	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Oilseed rape	Seedlings	Graner et al. 2003
	Potato	Plant tissues	De Boer and Copeman 1974
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998

Bacterial Endophyte	Plant	Organ	Reference
<i>Micrococcus</i>	Rice	Root	Germida and Siciliano 2001
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Micromonospora</i>	Cereal plants	Plant tissues	Coombs et al. 2003
<i>Moraxella</i>	Grapevine	Xylem	Bell et al. 1995
<i>Morganella</i>	Cotton	Plant tissues	Chen et al. 1995
	Rice	Seedlings	Mukhopadhyay et al. 1996
<i>Mycobacterium</i>	Scots pine	Bud	Mattila 2001
<i>Nocardia</i>	Citrus	Branch	Araujo et al. 2002
	Potato	Plant tissues	Garbeva et al. 2001
	Rice	Root	Germida and Siciliano 2001
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Nocardioides albus</i>	Cereal plants	Plant tissues	Coombs et al. 2003
<i>Ochrobacterium</i>	Corn	Stem	McInroy and Kloepper 1995
	Cotton	Root	McInroy and Kloepper 1995
	Rice	Root	Englehard et al. 2000
	Rice	Root	Germida and Siciliano 2001
<i>Pasteurella</i>	Potato	Tuber	Sturz et al. 1998
	Red clover	Stem	Sturz et al. 1997
<i>Phytobacterium</i>	Potato	Tuber	Sturz et al. 1998
<i>Phyllobacterium</i>	Clover	Root	Sturz et al. 1998
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Red clover	Nodule	Sturz et al. 1997
<i>Promicromonospora</i>	Potato	Tuber	Sturz et al. 1999
<i>Proteus</i>	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Providencia</i>	Citrus	Xylem	Gardner et al. 1982
<i>Psychrobacter</i>	Clover	Root	Sturz et al. 1998
	Potato	Tuber	Sturz et al. 1999
	Potato	Tuber	Sturz et al. 1998
<i>Rahnella</i>	Grapevine	Xylem	Bell et al. 1995
	Oilseed rape	Seedlings	Graner et al. 2003
	Pea	Stem	Elvira-Recueno & van Vuurde 2000
<i>Rathayibacter</i>	Canola	Root	Germida et al. 1998
	Rice	Root	Germida and Siciliano 2001
<i>Rhodococcus</i>	Cotton	Plant tissues	Chen et al. 1995
	Grapevine	Xylem	Bell et al. 1995
	Rice	Root	Germida and Siciliano 2001
<i>Rhodopseudomonas</i>	Rice	Plant tissues	Elbeltagy et al. 2000
<i>Rothia</i>	Corn, Sorghum	Aerial tissues	Zinniel et al. 2002
<i>Runella zeae sp. nov.</i>	Corn	Stem	Chelis et al. 2002
<i>Salmonella</i>	Cotton	Plant tissues	Chen et al. 1995
	Rice	Root	Germida and Siciliano 2001

Bacterial Endophyte	Plant	Organ	Reference
<i>Serratia</i>	Citrus	Xylem	Gardner et al. 1982
	Clover	Root	Sturz et al. 1998
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Potato	Tuber	Sturz et al. 1998
	Rice	Seedlings	Mukhopadhyay et al. 1996
	Yam	Tuber	Mantell 1998
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Shewanella</i>	Potato	Tuber	Sturz et al. 1998
<i>Shigella</i>	Citrus	Xylem	Gardner et al. 1982
<i>Sinorhizobium</i>	Potato	Plant tissues	Garbeva et al. 2001
<i>Sphingobacterium</i>	Potato	Tuber	Sturz et al. 1999
	Rice	Root	Germida and Siciliano 2001
<i>Sphingomonas</i>	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Root	McInroy and Kloepper 1995
	Elm	Stem, root	Mocali et al. 2001
	Potato	Tuber	Sturz et al. 1999
	Red clover	Root	Sturz and Christie 1998
	Rice	Plant tissues	Elbeltagy et al. 2000
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Streptococcus</i>	27 plant species	Ovule, seed	Mundt and Hinkle 1976
<i>Streptomyces</i>	Cereal plants	Plant tissues	Coombs et al. 2003
	Citrus	Branch	Araujo et al. 2002
	Corn	Leaf	de Araujo et al. 2000
	Laurel	Plant tissues	Nishimura et al. 2002
	Ryegrass	Plant tissues	Gurney and Mantle 1993
	<i>Kennedia nigriscans</i>	Plant tissues	Castillo-Uvidelio et al. 2002
	27 plant species	Ovule, seed	Mundt and Hinkle 1976
	Corn	Leaf	de Araujo et al. 2000
<i>Streptosporangium</i>	Corn	Leaf	de Araujo et al. 2000
<i>Variovorax</i>	Clover	Root	Sturz et al. 1998
	Corn	Stem, root	McInroy and Kloepper 1995
	Cotton	Root	McInroy and Kloepper 1995
	Potato	Tuber	Sturz et al. 1999
	Rice	Root	Germida and Siciliano 2001
	Citrus	Xylem	Gardner et al. 1982
	Corn	Stem	Fisher et al. 1992
<i>Vibrio</i>	Potato	Tuber	Sturz et al. 1998
<i>Yersinia</i>	Citrus	Xylem	Gardner et al. 1982
	Corn	Stem	McInroy and Kloepper 1995
	Cotton	Stem, root	McInroy and Kloepper 1995
	Cotton	Plant tissues	Chen et al. 1995
	Rice	Seedlings	Mukhopadhyay et al. 1996

Appendix 10. Plant bacterial endophytes with documented antagonism towards pathogens.

Bacterial Endophyte	Isolated From	Antagonism Property	Experiment Type	Plant	Pathogen	Reference
<i>Acidovorax delafieldii</i>	Oilseed rape	Antagonism	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003
<i>Acinetobacter</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Aerococcus</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Agrobacterium</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Agrobacterium rubi</i>	Oilseed rape	Antagonism	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003
<i>Agrobacterium tumefaciens</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Agrobacterium tumefaciens</i> B	Clover root	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Sturz et al. 1998
<i>Arthrobacter</i>	Potato tuber	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Arthrobacter ilicis</i>	Clover root	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Sturz et al. 1998
<i>Aureobacterium</i>	Potato tuber	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Aureobacterium saepidae</i> INR-6	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Bacillus</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Bacillus brevis</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Bacillus cereus</i> 65	<i>Sinapis</i>	Chitinase	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus cereus</i> 65	<i>Sinapis</i>	Chitinase	<i>In vitro</i>		<i>Pythium ultimum</i>	Pleban et al. 1995

Bacterial Endophyte	Isolated From	Antagonism Property	Experiment Type	Plant	Pathogen	Reference
<i>Bacillus cereus</i> 65	<i>Sinapis</i>	Chitinase	<i>In vitro</i>		<i>Sclerotium rolfsii</i>	Pleban et al. 1995
<i>Bacillus cereus</i> 65	<i>Sinapis</i>	Reduced disease	<i>In planta</i>	Cotton	<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus cereus</i> 65	<i>Sinapis</i>	Reduced disease	<i>In planta</i>	Bean	<i>Sclerotium rolfsii</i>	Pleban et al. 1995
<i>Bacillus cereus</i> 78	Cauliflower	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus cereus</i> 78	Cauliflower	Reduced disease	<i>In planta</i>	Bean	<i>Sclerotium rolfsii</i>	Pleban et al. 1995
<i>Bacillus licheniformis</i>	Oilseed rape	Protease	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003
<i>Bacillus mojaviensis</i>	Corn kernel	Antagonism	<i>In vitro</i>		<i>Fusarium moniliforme</i>	Bacon and Hinton 2002
<i>Bacillus pumilus</i> 85	Sunflower	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus pumilus</i> 85	Sunflower	Antagonism	<i>In vitro</i>		<i>Sclerotium rolfsii</i>	Pleban et al. 1995
<i>Bacillus pumilus</i> 85	Sunflower	Reduced disease	<i>In planta</i>	Cotton	<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus pumilus</i> JM-1128	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Bacillus pumilus</i> SE34	Endophyte	Induced defense-related ultrastructural modifications	<i>In planta</i>	Pea	<i>Fusarium oxysporum</i> f. sp. <i>pisi</i>	Benhamou et al. 1996a
<i>Bacillus subtilis</i> 72	Onion tissue	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus subtilis</i> 72	Onion tissue	Antagonism	<i>In vitro</i>		<i>Pythium ultimum</i>	Pleban et al. 1995
<i>Bacillus subtilis</i> 72	Onion tissue	Reduced disease	<i>In planta</i>	Cotton	<i>Rhizoctonia solani</i>	Pleban et al. 1995
<i>Bacillus subtilis</i> 72	Onion tissue	Reduced disease	<i>In planta</i>	Bean	<i>Sclerotium rolfsii</i>	Pleban et al. 1995
<i>Brevibacterium</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Brochothrix</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995

Bacterial Endophyte	Isolated From	Antagonism Property	Experiment Type	Plant	Pathogen	Reference
<i>Burkholderia</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Burkholderia (Pseudomonas) cepacia</i>	Asparagus	Mycelia deformation	<i>In planta</i>	Banana	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> race 4	Pan et al. 1997
<i>Burkholderia solanacearum</i> JM-869	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Cedeca davisae</i> MK-30	Endophyte	Possible ISR	<i>In planta</i>	Tomato	<i>Meloidogyne incognita</i> (nematode)	Munif et al. 2001
<i>Cedecea</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Chryseomonas</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Citrobacter</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Clavibacter</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Corynebacterium</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Curtobacterium flaccumfaciens</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Curtobacterium</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Curtobacterium albidum</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Curtobacterium flaccumfaciens</i>	Clover root Potato tuber	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Sturz et al. 1998
<i>Curtobacterium luetum</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Cytophaga johnsonae</i>	Oilseed rape	Protease	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003

Bacterial Endophyte	Isolated From	Antagonism Property	Experiment Type	Plant	Pathogen	Reference
<i>Enterobacter</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Enterobacter (Pantoea) agglomerans</i>	Clover root	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Sturz et al. 1998
<i>Enterobacter cloacae</i>	Potato tuber					
	Spinach root	Antagonism	<i>In planta</i>	Spinach	<i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i>	Tsuda et al. 2001
<i>Enterobacter</i> sp. MK-42	Endophyte	Possible ISR	<i>In planta</i>	Tomato	<i>Meloidogyne incognita</i> (nematode)	Munif et al. 2001
<i>Erwinia</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Flavimonas</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Flavobacterium</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Hydrogenophaga</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Klebsiella pneumonia</i>	Clover root	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Sturz et al. 1998
<i>Methylobacterium</i>	Potato tuber					
	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Microbacterium</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Micrococcus</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Micrococcus varians</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Morganella</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Nocardioides albus</i>	Cereal	Antagonism	<i>In planta</i>	Wheat	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Coombs et al. 2003

Bacterial Endophyte	Isolated From	Antagonism Property	Experiment Type	Plant	Pathogen	Reference
<i>Paenibacillus polymyxa</i>	Oilseed rape	Protease	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003
<i>Pantoea agglomerans</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Erwinia cartovora</i> var. <i>atroseptica</i>	Struz and Matheson 1996
<i>Pantoea agglomerans</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Pantoea agglomerans</i> MK-29	Endophyte	Possible ISR	<i>In planta</i>	Tomato	<i>Meloidogyne incognita</i> (nematode)	Munif et al. 2001
<i>Phyllobacterium</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Phyllobacterium rubiacearum</i> JM-1137	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Pseudomonas</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Pseudomonas cichorii</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Pseudomonas cichorii</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Fusarium avenaceum</i> , <i>oxysporum</i> , <i>sambucinum</i>	Sturz et al. 1999
<i>Pseudomonas corrugata</i>	Clover root Potato tuber	Antagonism	<i>In vitro</i>		<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	Sturz et al. 1998
<i>Pseudomonas corrugata</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Pseudomonas corrugata</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Fusarium avenaceum</i> , <i>oxysporum</i> , <i>sambucinum</i>	Sturz et al. 1999
<i>Pseudomonas fluorescens</i>	Oilseed rape	Antagonism	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003
<i>Pseudomonas fluorescens</i> S3	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Achlya klebsiana</i>	Adhikari et al. 2001
<i>Pseudomonas fluorescens</i> S3	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Pythium spinosum</i>	Adhikari et al. 2001
<i>Pseudomonas putida</i>	Oilseed rape	Protease	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003

Bacterial Endophyte	Isolated From	Antagonism Property	Experiment Type	Plant	Pathogen	Reference
<i>Pseudomonas putida</i> 1-15	Live Oak	Antagonism	<i>In planta</i>	Live oak	<i>Ceratocystis fagacearum</i>	Brooks et al. 1994
<i>Pseudomonas putida</i> 5-48	Live Oak	Antagonism	<i>In planta</i>	Live oak	<i>Ceratocystis fagacearum</i>	Brooks et al. 1994
<i>Pseudomonas putida</i> 89B-61	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Pseudomonas putida</i> CC-186	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Pseudomonas putida</i> MT-19	Endophyte	Possible ISR	<i>In planta</i>	Tomato	<i>Meloidogyne incognita</i> (nematode)	Munif et al. 2001
<i>Pseudomonas talaasii</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Pseudomonas tolaasi</i> S20	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Achlya klebsiana</i>	Adhikari et al. 2001
<i>Pseudomonas tolaasi</i> S20	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Pythium spinosum</i>	Adhikari et al. 2001
<i>Pseudomonas tolaasii</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Fusarium avenaceum</i> , <i>oxysporum</i> , <i>sambucinum</i>	Sturz et al. 1999
<i>Pseudomonas veronii</i> S21	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Achlya klebsiana</i>	Adhikari et al. 2001
<i>Pseudomonas veronii</i> S21	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Pythium spinosum</i>	Adhikari et al. 2001
<i>Rahnella aquatilis</i>	Oilseed rape	Antagonism	<i>In vitro</i>		<i>Verticillium longisporum</i>	Graner et al. 2003
<i>Rhizobium meliloti</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1, A2	Sturz et al. 1999
<i>Rhodococcus</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Salmonella</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Serratia</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995

Bacterial Endophyte	Isolated From	Antagonism Property	Experiment Type	Plant	Pathogen	Reference
<i>Serratia plymuthica</i>	Endophyte	Induced resistance	<i>In planta</i>	Cucumber	<i>Pythium ultimum</i>	Benhamou et al. 2000
<i>Sphingomonas paucimobilis</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Phytophthora infestans</i> A1&2	Sturz et al. 1999
<i>Sphingomonas trueperi</i> S12	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Achlya klebsiana</i>	Adhikari et al. 2001
<i>Sphingomonas trueperi</i> S12	Endophyte	Antagonism	<i>In planta</i>	Rice	<i>Pythium spinosum</i>	Adhikari et al. 2001
<i>Staphylococcus</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Streptomyces bikiniensis</i>	Cereal plants	Antagonism	<i>In planta</i>	Wheat	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Coombs et al. 2003
<i>Streptomyces caviscabies</i>	Cereal plants	Antagonism	<i>In planta</i>	Wheat	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Coombs et al. 2003
<i>Streptomyces galilaeus</i>	Cereal plants	Antagonism	<i>In planta</i>	Wheat	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Coombs et al. 2003
<i>Streptomyces</i> NRRL3052	<i>Kennedia nigricans</i>	Munumbicins A-D antibiotics	<i>In vitro</i>		Did not state.	Castillo et al. 2002
<i>Streptomyces</i> sp.	Perennial ryegrass	1-N-methylalbonoursin	<i>In vitro</i>		Did not state.	Gurney and Mantel 1993
<i>Streptomyces</i> sp. AOK-30	Mountain Laurel	Antimicrobial	<i>In planta</i>	Laurel	<i>Pestalotiopsis sydneyana</i>	Nishimura et al. 2002
<i>Streptomyces argenteolus</i>	Cereal	Antagonism	<i>In planta</i>	Wheat	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Coombs et al. 2003
<i>Xanthomonas</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995
<i>Xanthomonas campestris</i>	Clover root	Antagonism	<i>In vitro</i>		<i>Rhizoctonia solani</i>	Sturz et al. 1998
<i>Xanthomonas oryzae</i>	Potato tuber	Antagonism	<i>In vitro</i>		<i>Fusarium avenaceum</i> , <i>oxysporum</i>	Sturz et al. 1999
<i>Yersinia</i>	Cotton	Antagonism	<i>In planta</i>	Cotton	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Chen et al. 1995

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VITA

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Doctor of Philosophy

Thesis: CULTURABLE BACTERIAL ENDOPHYTES FROM BERMUDAGRASS
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HERPOTRICHA

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Scope and Method of Study: There are several goals of this study. First was to document and putatively identify the culturable bacterial endophytes isolated from surface sterilized crown tissue of Midlawn and Tifgreen cultivars of bermudagrass, secondly, to test these endophytes for antagonistic properties against the Spring Dead Spot (SDS) soilborne fungal pathogen, *Ophiostoma herpotricha*, and thirdly, to identify promising candidates for a biological control agent against SDS. Another major goal was to develop a real-time quantitative PCR assay for *O. herpotricha* using TaqMan[®] chemistry and use this assay to document the spatial distribution of *O. herpotricha* infection in plant and soil samples and document the relationship between resistant and susceptible cultivars of bermudagrass and SDS.

Findings and Conclusions: Seventy-seven Gram-negative and 51 Gram-positive culturable bacterial endophytes, including 31 with *in vitro* antifungal attributes, were readily isolated from the crown tissue of Midlawn and Tifgreen cultivars of bermudagrass, infected with *O. herpotricha* and non-infected. This study is the first, to my knowledge, to document a *Geodermatophilus* sp. and a *Amycolatopsis* sp. as plant endophytes, and of *in vitro* antifungal attributes of a *Chryseobacterium* sp. The abundance and diversity of culturable bacterial endophytes in bermudagrass demonstrate that turfgrasses are good hosts and valuable resources for endophytes with antifungal properties. The cohort of *in vitro* antifungal bacterial endophytes has potential as biological control agents for SDS. A standard-curve real-time quantitative PCR assay with TaqMan[®] chemistry was developed to identify and quantify the DNA levels of *O. herpotricha* in plant and soil samples. This assay has proven to be quantitative, sensitive, selective, rapid, and easy to perform and may lead to its application to the study of other plant diseases.

ADVISER'S APPROVAL: Michael P. Anderson